KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOTECHNOLOGY

I. M.Sc. BIOTECHNOLOGY (SEMESTER –II)

FERMENTATION AND BIOPROCESS TECHNOLOGY (19BTP202)

SYLLABUS

UNIT –I Introduction:

Isolation and screening of industrially important strains- primary and secondary screening. Strain improvement, mutation, selection of mutants, recombination – bacteria, fungi and actinomycetes, assay and fermented products. Fermentations - submerged, solid state.

UNIT – II Media:

Media formulation – sterilization – batch and continuous sterilization, sterilization of air, fibrous filters. Microbial kinetics: batch, fed-batch and continuous cultures, phases of batch growth. kinetics of cell growth, product formation, substrate utilization, product inhibition kinetics, yield concept and productivity.

UNIT – III Design of fermenter:

Types – CSTR, Tower, jet loop, air lift fermenter, bubble column, packed bed. Fundamentals of process control and monitoring – on line and off line analysis, feedback control, PID controller, computer aided control.

UNIT – IV Kinetics:

Transport phenomena – Rheological properties, determination of O2 mass transfer, heat transfer, role of aeration and agitation, factors affecting O2 transfer. Production of chemicals – alcohol, antibiotics – Penicillin and Streptomycin, Single cell proteins.

UNIT -V Downstream processing:

Cell distribution methods for intracellular products; foam separation, precipitation. Filtration – micro and ultra-filtration; Solvent extraction-, chromatographic separation- FPLC, HPLC, dialysis, centrifugation, distillation, drying, crystallization, turbidity analysis and cell yield determination. Fermentation products – available in market.

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I. M.Sc. BIOTECHNOLOGY (SEMESTER – II)

LECTURE PLAN- FERMENTATION AND BIOPROCESS TECHNOLOGY (19BTP202)

S. No.	Lecture duration (Hr)	Topic to be covered	Support materials/ Page no.
UNIT - I			
1	1	Isolation and screening of industrially important strains	T1:1 -3; 35-40; W1
2	1	Primary and secondary screening	T1: 40-42
3	1	Strain improvement, mutation, selection of mutants	T1: 43-48
4	1	Recombination of bacteria, fungi and actinomycetes	T1: 70-75
5	1	Assay and fermented products	T1: 14-18
6	1	Fermentation- submerged, solid state	T1: 14-18
7	1	Revision	
UNIT - II			
8	1	Media formulation	T1: 93-116
9	1	Sterilisation-batch & continuous sterilisation, sterilisation of air, fibrous filters	T1: 123-141
10	1	Microbial kinetics: Batch, fed batch and continuous cultures, phases of batch growth	T1: 17-22
11	1	Kinetics of cell growth, product formation	T1: 22-26
12	1	Substrate utilisation, product inhibition kinetics	T1: 22-26
13	1	Yield concept and productivity	T1: 13-17
14	1	Revision	
UNIT – III			
15	1	Types- CSTR, Tower, Jet loop	T1: 167-176
16	1	Airlift fermenter, bubble column, packed bed.	T1: 200-210
17	1	Fundamentals of process control and monitoring	T1: 215-225
18	1	Online and off line analysis	T1: 228-232
19	1	Feedback control, PID control	T1: 228-232
20	1	Computer aided control	T1: 234-237

21	1	Revision	
UNIT – IV			
22	1	Transport phenomena	T1: 252-254
23	1	Rheological properties	
24	1	Determination of O2 mass transfer, Heat transfer	T1-: 108-109
25	1	Role of aeration and agitation	T1: 178-180
26	1	Factors affecting O2 transfer, RSM	
27	1	Production of chemicals- alcohol	T2: 112-172
28	1	Antibiotics	
29	1	Penicillin and streptomycin	
30	1	Single cell Proteins	T2: 178-187
31	1	Revision	
UNIT – V			
32	1	Cell distribution methods for intracellular products	T1: 292-296
33	1	Foam separation, precipitation	T1: 280-285
34	1	Filtration- micro and ultrafiltration	
35	1	Solvent extraction	T1: 296- 304
36	1	Chromatographic separation – FPLC, HPLC	
37	1	Dialysis, centrifugation, distillation, drying crystallisation	T1: 287-290
38	1	Turbidity analysis and cell yield determination	T1: 304-308
39	1	Fermentation products available in market	T1: 334-336
40	1	Revision	

Textbooks

T1: P.R.Stanbery, A. Whitaker and S.J. Hall. (2006). Principles of fermentation technology, Elsevier Science Ltd.

T2: A.H. Patel (2007). Industrial Microbiology, Macmillan India Ltd.

Website

W1: http://www.scribed.com/doc/219/0402/industrial-microbiology.

UNIT –I Introduction:

Isolation and screening of industrially important strains- primary and secondary screening. Strain improvement, mutation, selection of mutants, recombination – bacteria, fungi and actinomycetes, assay and fermented products. Fermentations - submerged, solid state.

Introduction

Fermentation is the chemical transformation of organic substances into simpler compounds by the action of enzymes, complex organic catalysts, which are produced by microorganisms such as molds, yeasts or bacteria. Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (and in the case of foods more easily digestible) compounds and nutrients.

- For example, the enzyme protease breaks down huge protein molecules first into polypeptides and peptides, then into numerous amino acids, which are readily assimilated by the body.
- The enzyme amylase works on carbohydrates, reducing starches and complex sugars to simple sugars.
- And the enzyme lipase hydrolyzes complex fat molecules into simpler free fatty acids.

These are but three of the more important enzymes. There are thousands more, both inside and outside of our bodies. In some fermentations, important by-products such as alcohol or various gases are also produced. The word "fermentation" is derived from the Latin "*ferever*" meaning "to boil," since the bubbling and foaming of early fermenting beverages seemed closely akin to boiling.

People have known about and used fermentation for thousands of years. But it has been only in the past two hundred years that scientists have come to understand this important process. In 1854, the French chemist Louis Pasteur determined that fermentation is caused by yeast. His work was influenced by the earlier work of Theodor Schwann, the German scientist who helped develop the cell theory. Around 1840, Schwann concluded that fermentation is the result of processes that occur in living things.

Wang and Hesseltine (1979) note that "Probably the first fermentation were discovered accidentally when salt was incorporated with the food material, and the salt selected certain harmless microorganisms that fermented the product to give a nutritious and acceptable food." The process was taken a step further by the early Chinese who first inoculated with the basic foods with molds, which created enzymes; in salt-fermented soy foods such as miso, soy sauce and soy nuggets.

The chronological development of the fermentation industry

The chronological development of the fermentation industry may be represented as five overlapping stages

In the late 1700s Lavoisier showed that in the process of transforming sugar to alcohol and carbon dioxide (as in wine), the weight of the former that was consumed in the process equaled the weight of the latter produced. In 1810 J.L. Guy-Lussac summarized the process with the famous equation $C_6H_{12}O_6$ yields $2CO_2$ + 2 C_2H_6O. The entire process was considered to be simply a chemical reaction and yeast (which was not yet even classified as a definite substance, much less a living organism instrumental to fermentation) was thought to play a physical rather than a chemical role, an idea dating back to the time of George Stahl in 1697. It was held that either the catalytic action at the yeast cell or the molecular vibrations from the decomposing

organic matter arising from the death of the cells, sparked the chemical changes resulting in fermentation. Putrefaction, spoilage, and fermentation were all considered to be processes of death, not life.

Stage	Main products	Vessels	Process control	Culture method	Quality control	Pilot plant facilities	Strain selection
1 Pre-1900	Alcohol	Wooden, up to 1500 barrels capacity Copper used in later breweries	Use of thermometer, hydrometer and heat exchangers	Batch	Virtually nil	Nil	Pure yeast cultures used at the Carlsberg brewery (1886)
	Vinegar	Barrels, shallow trays, trickle filters		Batch	Virtually nil	Nil	Fermentations inoculated with 'good' vinegar
2 1900–1940	Bakers' yeast glycerol, citric acid, lactic acid and acetone/ butanol	Steel vessels of up to 200 m^3 for acetone/butanol Air spargers used for bakers' yeast Mechanical stirring used in small vessels	pH electrodes with off-line control Temperature control	Batch and fed-batch systems	Virtually nil	Virtually nil	Pure cultures used
3 1940–date	Penicillin, streptomycin, other antibiotics, gibberelin, amino acids, nucleotides, transformations, enzymes	Mechanically aerated vessels, operated aseptically—true fermenters	Sterilizable pH and oxygen electrodes. Use of control loops which were later computerized	Batch and fed-batch common Continuous culture introduced for brewing and some primary metabolites	Very important	Becomes common	Mutation and selection programmes essential
4 1964–date	Single-cell protein using hydrocarbon and other feedstocks	Pressure cycle and pressure jet vessels developed to overcome gas and heat exchange problems	Use of computer linked control loops	Continuous culture with medium recycle	Very important	Very important	Genetic engineering of producer strains attempted
5 1979–date	Production of heterologous proteins by microbial and animal cells Monoclonal antibodies produced by animal cells	Fermenters developed in stages 3 and 4. Animal cell reactors developed	Control and sensors developed in stages 3 and 4	Batch, fed-batch or continuous Continuous perfusion developed for animal cell processes	Very important	Very important	Introduction of foreign genes into microbial and animal cell hosts. *In vitro* recombinant DNA techniques used in the improvement of stage 3 products

The early 1800s saw a great increase of interest in microbiology in Europe. The scientific period began with great advances in botany, increased interest in microscopy, and willingness to investigate individual organisms. The two major problems that would challenge the greatest researchers in the new field of microbiology concerned the basic nature of the fermentation process and the basic nature of enzymes. The scientific breakthroughs that would lead to the

unraveling of the mysteries of fermentation starting in the 1830s were made primarily by French and German chemists.

The debate was finally brought to an end by the great French chemist Louis Pasteur (1822-1895) who, during the 1850s and 1860s, in a series of classic investigations, proved conclusively that fermentation was initiated by living organisms. In 1857 Pasteur showed that lactic acid fermentation is caused by living organisms. In 1860 he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization.

In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, *Etudes sur la Biere* , which was translated into English in 1879 as *Studies on Fermentation* . He defined fermentation (incorrectly) as "Life without air," but correctly showed specific types of microorganisms cause specific types of fermentations and specific end products.

Many scientists, including Pasteur, had attempted unsuccessfully to extract the fermentation enzyme from yeast. Success came finally in 1897 when the German chemist Eduard Buechner ground up yeast, extracted a juice from them, then found to his amazement that this "dead" liquid would ferment a sugar solution, forming carbon dioxide and alcohol, just like living yeasts. Clearly the so-called "unorganized ferments" behaved just the organized ones. From that time on the term "enzyme" came to be applied to all ferments.

The term "ferment" dropped out of the scientific vocabulary altogether and the vitalist position collapsed, never to recover. Thereafter it was agreed that only one set of laws applied to all things, both animate and inanimate and that there was no special vital force which

characterized living things and acted under different laws. And it was finally understood that fermentation is caused by enzymes which are produced by microorganisms.

In 1907, a German chemist named Eduard Buchner received the Nobel prize for showing that enzymes in yeast cells cause fermentation. About two decades later, two other scientists determined exactly how enzymes cause fermentation. Their names are Arthur Harden and Hans Euler-Chelpin, and they won the Nobel prize for their work in 1929. By the 1940s, technology was developed to use fermentation to produce antibiotics.

The sciences of microbiology, biochemistry, fermentation technology, mycology, and bacteriology all shared a deep interest in the nature and working of enzymes. Yet still by the early 1900s no one knew exactly what enzymes were or how they acted. As the agricultural microbiologist Conn asked in 1901, "How can they produce chemical actions without being acted upon or entering into the reactions? Are enzymes fully lifeless or semi-living? We still do not know the fundamental mystery of fermentation." Gradually an understanding of enzymes and catalysts developed. In 1905 Harden and Young discovered coenzymes, agents necessary for the action of enzymes. In 1926 the American biochemist J.B. Sumner first purified and crystallized an enzyme (urease) and showed that it was a protein, more precisely a protein catalyst. Eventually enzymes came to be seen as the key catalysts in all the life processes, each highly specialized in its catalytic action and generally responsible for only one small step in complex, multi-step biochemical reactions.

Advances in microbiology and fermentation technology have continued steadily up until the present. For example, in the late 1930s it was discovered that microorganisms could be mutated with physical and chemical treatments to be higher yielding, faster growing, tolerant of

less oxygen, and able to use a more concentrated medium. Strain selection and hybridization developed as well, affecting most modern food fermentations (Hesseltine and Wang 1977).

Since ancient times the koji making process has been unique to East Asia, where it has been used in the preparation of fermented foods such as miso, soy sauce, soy nuggets, sake, shochu (spirits), and rice vinegar (*yonezu*). Some of the scientist suggested that molds are widely used since they grow well in areas having a humid climate and long rainy season during the warm months. In the West mold fermented foods are limited primarily to a number of cheeses characterized by their strong flavors and aromas: Camembert, Blue, Brie, and related types. Because of the widespread use of mold-fermented foods in East Asia, the word "mold" there has a rather positive connotation, something like "yeast" in the West. Most Westerners still have a deep-seated prejudice against moldy products, and they generally associate the word "mold" with food spoilage, as in "moldy bread". The Chinese had distinct names for two types of molds used in fermented soyfoods; what we now call *Aspergillus* was then called "yellow robe" and *Rhizopus* was called "white robe." These cultures were carefully distinguished and propagated from year to year. By the 10th century a koji starter or inoculum was deliberately being used in the preparation of koji for fermented foods (Tamiya 1958; Sakaguchi 1972; 1979).

During the 20th century, Japanese microbiologists have made many important contributions to the development of applied and industrial microbiology, including the manufacture of fermented soyfoods, as well summarized by Tamiya (1958) and Sakaguchi (1972). Until quite recently, their strength was more in the area of application of scientific knowledge than in pioneering basic scientific and microbiological breakthroughs. From the early 1900s, important studies on the koji mold and its enzymes were done by Japanese scientists. Important advances in enzymology, with much of the work done on koji molds, began in the

1920s. In 1928 Miyazaki developed the combined Amylo-Koji process. By the 1950s Japanese scientists had isolated various protease and amylase enzymes, induced mutations, and used them commercially. They also developed the technology for the microbial production of L-glutamic acid and monosodium glutamate (MSG), lysine and other amino acids, flavor enhancing nucleotides such as inosinic acid, and organic acids. They used the koji mold *Aspergillus oryzae* in the commercial production of enzymes including proteases, amylases, amyloglucosidase, and lipase. They made microbial rennet and numerous other products. Indeed in the period following World War II, Japan became the world leader in the field of industrial fermentations. Wang and Hesseltine (1979) have suggested that this may have been "in large part due to the food fermentation base from which it launched its industrialization of micoorganisms."

According to Tamiya, in 1958 food and drinks produced with koji retailed for $1,000 million a year, and the taxes from these foods amounted to more than $500 million, which was as much as 20% of the Japanese national budget! In 1970 in Japan, foods made from koji molds accounted for 1.5% of the nation's Gross National Product, or about $205,000 million. Prominent among these were miso and shoyu (Sakaguchi 1972). Production of fermented soyfoods continues to be the most important of the fermented food industries of East Asia. The many important developments in this field will be described in the following chapters.

Starting in about the 1960s and increasing rapidly after the mid-1970s, East Asian fermented soyfoods (especially soy sauce or shoyu, miso, and tempeh, in that order), began to be widely used in the West. Reasons for this include the growing general interest in soyfoods, the cultural and religious movement toward meatless and vegetarian diets, the increasing interest in nutritious foods with less animal fats, the awareness these foods as a good vegetarian source of

vitamin B-12, the growing worldwide travel stimulating interest in foreign foods, the increase of East Asian refugees to the West, and the increased interest in microbiology and enhanced image of fermented foods. All of these developments indicate a bright future for fermented soyfoods in the West (Hesseltine 1981).

The use of microbes to obtain a product or service of economic value constitutes industrial microbiology. Any process mediated by or involving microorganisms in which a product of economic value is obtained is called fermentation (Casida, Jr., 1968).

The terms industrial microbiology and fermentation are virtually synonymous in their scope, objectives and activities.

The microbial product may be microbial cells (living or dead), microbial biomass (the total quantity or weight of organisms in a given area or volume), and components of microbial cells, intracellular or extracellular enzymes or chemicals produced by the microbes utilizing the medium constituents or the provided substrate.

The services generated by microorganisms range from the degradation of organic wastes, detoxification of industrial wastes and toxic compounds, to the degradation of petroleum to manage oil spills, etc. Industrial microbiology also encompasses activities like production of biocontrol agents, inoculants used as biofertilizers, etc.

Obviously, the scope and activities of industrial microbiology are too extensive to be covered in any detail in a book like this scope; therefore, the coverage in this chapter remains generalized and rather elementary.

The activities in industrial microbiology begin with the isolation of microorganisms from nature, their screening for product formation, improvement of product yields, maintenance of cultures,

mass culture using bioreactors, and usually end with the recovery of products or metabolites and their purification.

The metabolism may be defined as the sum of the physical and chemical processes in an organism by which its substance is produced, maintained, and destroyed, and by which energy is made available.

Microbial Products of Potential Importance

Product / Activity	**Examples**
Products	
1. Amino acids	L-glutarnic acid, L-lysine
2. Antibiotics	Streptomycin, penicillin, tetracyc1ines, polymyxin
3. Beverages	Wine, beer, distilled beverages
4. Biodegradable plastic	β-polyhydroxybutyrate
5. Enzymes	Amylase, proteases, pectinases, invertase, cellulase
6. Flavouring agents	Monosodium glutamate, nucleotides
7. Foods	Cheese, pickles, yoghurt, bread, vinegar
8. Gases	CO2, H2,CH4
9. Organic acids	Lactic, citric, acetic, butyric, fumaric
10. Organic solvents	Acetone, ethanol, butanol, amyl alcohol
11. Others	Glycerol, fats, steroids, gibberellins
11 a. Vitamins	B12, riboflavin, A
12. Recombinant proteins	Insulin, interferon, subunit vaccines
13. Substrates	A wide range of compounds used for chemical syntheses of valuable products.

Cells/Biomass

14. Biomass	Food and feed yeast, other organisms used as single cell protein (SCP)
15. Cells	Biofertilizers, biocontrol agents, bacterial insecticides, mycorrhyzae
16. Vaccines	A variety of viral and bacterial vaccines

Activities

Biotransformation	Steroids, antibiotics D-sorbitol
Degradation	Disposal of biological and industrial wastes, detoxification of toxic compounds, petroleum
Solubilization/accumulation	Improved recovery of oil and metals, discovery of new oil reserves, removal of toxic metals

List of microbes for the production of antibiotics and Commercial Activities of the antibiotics

Antibiotic	**Produced by**	**Activity**
Cephalosporin C	*Cephalosporium acremonium*	Gram + and - bacteria
Gentamycin	*Micromonospora purpurea*	Gram+ bacteria
Griseofulvin	*Penicillium griseofulvum*	Gram+ and - bacteria
Kanamycin	*S. kanamyceticus*	Gram+ and - bacteria and mycobacteria
Neomycins	*S. fradiae*	Gram+ and - bacteria
Pimaricin	*S. natalensis*	Antitumour

Penicillin G	*P. chrysogenum*	Gram+ bacteria
Polymixin B	*Bacillus polymyxa*	Antifungal Peptide
Streptomycin	*S. griceus*	Gram+ and - bacteria, and mycobacteria
Tetracyclines	*Streptomyces spp*	Gram+ and - bacteria

Important uses of industrial micro-organisms

Product	**Micro-organism**	**Use**
Vitamin B12	Streptomyces	vitamin supplements
Lactic Acid	Lactobacillius delbrueckii	chemical reagents
Citric Acid	Aspergillus niger	food preservative
Ethanoic Acid	Acetobacter sp.	vinegar, solvent
Pectinases Micriorganism	Aspergillus sp.	degrading pectin
Ethanol	Saccharomyces	chemical reagents, drinks
Penicillin	Penicillin chrysogenum	Antibiotic

List of microbes for the production of Enzymes and Commercial Activities of the enzymes

Enzyme	**Source**	**Application**	**Use in the Form**
α - Amylase	1. *Bacillius licheniformis* 2. *B.amylolique* faciens	Hydrolysis of starch to dextrans	Extracellular, soluble
Glucoamylase	*Aspergillus niger*	Dextran hydrolysis to glucose	Extracellular, soluble
Xylose (Glucose)	*Bacillus coagulans*	Pure glucose to equilibrium	Immobilized

isomerase		mixture of glucose + fructose	whole cells
Alkaline Protease	*B. licheniformis*, *B.subtilis*	Detergents (protein digestion)	Extracellular, soluble
Neutral Protease	*B.amylolique faciens*, *B.subtilis*	Protein digestion in brewing substances	Extracellular, soluble
Acid Protease	*A. niger*	Milk coagulation and cheese flavour enhancement	Extracellular, soluble
Pectinase	*A. niger*, *B.subtilis*	Pectin hydrolysis in fruit juices	Extracellular, soluble
Glucanase B.subtilis	*A. niger*,	In fruit juices	Extracellular, soluble
Cellulase	*A.niger*,	Trichoderma reesei Cellulose hydrolysis in fruit juices	Extracellular, soluble
Lipase	*Rhizopus Species*	Detergents, lipid hydrolysis	Extracellular, soluble
Lactase	*A.niger*	Milk lactose hydrolysis to glucose	Immobilized on glass beads

The range of fermentation processes

There are five major groups of commercially important fermentations:

(i) Those that produce microbial cells (or biomass) as the product.

(ii) Those that produce microbial enzymes.

(iii) Those that produce microbial metabolites.

(iv) Those that produce recombinant products.

(v) Those that modify a compound which is added to the fermentation the transformation process.

Microbial biomass

The commercial production of microbial biomass may be divided into two major processes: the production of yeast to be used in the baking industry and the production of microbial cells to be used as human or animal food (single-cell protein).

Microbial enzymes

Enzymes have been produced commercially from plant, animal and microbial sources. However, microbial enzymes have the enormous advantage of being able to be produced in large quantities by established fermentation techniques.

Microbial metabolites

The growth of a microbial culture can be divided into a number of stages. After the inoculation of a culture into a nutrient medium there is a period during which growth does not appear to occur; this period is referred to as the lag phase and may be considered as a time of adaptation. Following a period during which the growth rate of the cells gradually increases the cells grow at a constant, maximum rate and this period is known as the log, or exponential, phase. Eventually, growth ceases and the cells enter the so-called stationary phase. After a further period of time the viable cell number declines as the culture enters the death phase.

Many products of primary metabolism are of considerable economic importance and are being produced by fermentation.

During the deceleration and stationary phases some microbial cultures synthesize compounds which are not produced during the trophophase and which do not appear to have any obvious function in cell metabolism. These compounds are referred to as the secondary compounds of metabolism.

Recombinant product

The advent of recombinant DNA technology has extended the range of potential fermentation products. Genes from higher organisms may be introduced into microbial cells such that the recipients are capable of synthesizing 'foreign' (or heterologous) proteins. A wide range of microbial cells have been used as hosts for such system including Escherichia coli, Saccharomyces and filamentous fungi. Products produced by such genetically engineered organisms include interferon, insulin, human serum albumin, factors VIII and IX, epidermal growth factor, calf chymosin and bovine somatostatin.

Transformation processes

Microbial cells may be used to convert a compound into a structurally related, financially more valuable, compound. Because micro-organisms can behave as chiral catalysts with high positional specificity and stereospecificity. microbial processes are more specific than purely chemical ones and enable the addition, removal or modification of functional groups at specific Sites on a complex molecule without the use of chemical protection. The reactions which may be catalysed include dehydrogenation, oxidation, hydroxylation, dehydration and condensation, decarboxylation, amination, deamination and isomerization. Microbial processes have the additional advantage over chemical reagents of operating at relatively low temperatures and pressures without the requirement for potentially polluting heavy-metal catalysts.

The component parts of a fermentation process

Regardless of the type of fermentation (with the possible exception of some transformation processes) an established process may be divided into six basic component parts:

(i) The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermenter.

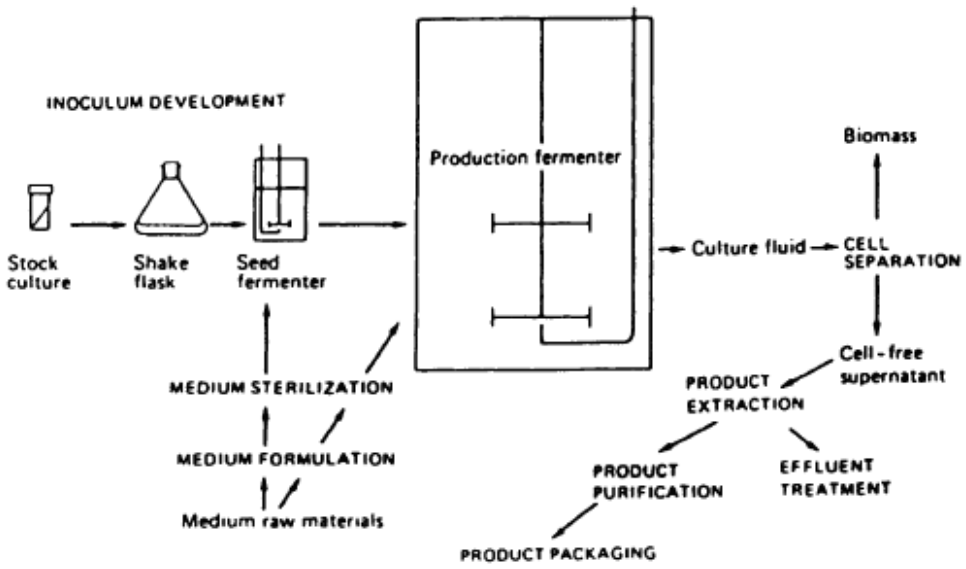
(ii) The sterilization of the medium, fermenters and ancillary equipment.

(iii) The production of an active, pure culture in sufficient quantity to inoculate the production vessel.

(iv) The growth of the organism in the production fermenter under optimum conditions for product formation.

(v) The extraction of the product and its purification.

(vi) The disposal of effluents produced by the process.



A generalized, schematic representation of a fermentation process

Fermentation

Concepts of fermentation

- The central component of the system is obviously the fermenter itself, in which the organism is grown under conditions optimum for product formation, one must not lose sight of operations upstream and downstream of the fermenter.

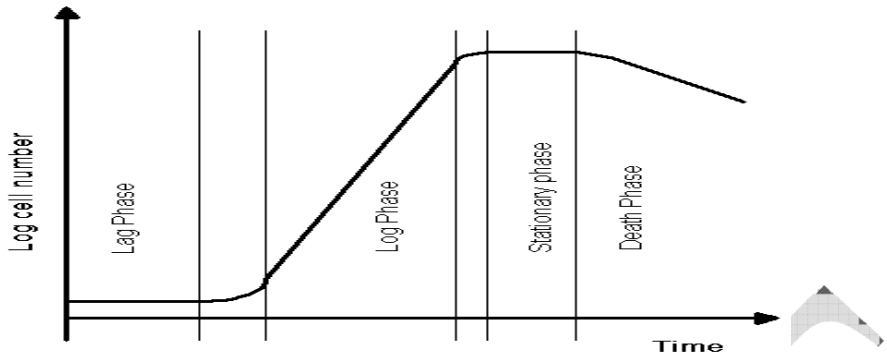
- Before the fermentation is started the medium must be formulated and sterilized, the fermenter sterilized, and a starter culture must be available in sufficient quantity and in the correct physiological state to inoculate the production fermenter.
- Downstream of the fermenter the product has to be purified and further processed and the effluents produced by the process have to be treated.

Various types of fermentation based on the culturing microbes

1. Batch fermentation
2. Fed-batch fermentation
3. Continuous

1. Batch fermentation

- A **batch fermentation** can be considered to be a closed system i.e. Fermentation conducted from start to end in a single vessel.
- At time t=0 the sterilized nutrient solution in the fermentor is **inoculated** with microorganisms and incubation is allowed to proceed.
- In the course of the entire fermentation, nothing is added, except oxygen (in case of aerobic microorganisms), an antifoam agent, and acid or base to control the pH. The composition of the culture medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of the metabolism of the cells.
- After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, four typical phases of growth are observed as indicated in below mentioned Figure.



Growth curve of a bacterial culture.

Lag phase

- Physicochemical equilibration between microorganism and the environment following inoculation with very little growth.

Log phase

- By the end of the lag phase cells have adapted to the new conditions of growth.
- Growth of the cell mass can now be described quantitatively as a doubling of cell number per unit time for bacteria and yeast's, or a doubling of biomass per unit time for filamentous organisms as fungi.
- By plotting the number of cells or biomass against time on a semilogarithmic graph, a straight line results, hence the term log phase.
- Although the cells alter the medium through uptake of substrates and excretion of metabolic products, the growth rate remains constant during the log phase.
- Growth rate is independent of substrate concentration as long as excess substrate is present.

Stationary phase

- As soon as the substrate is metabolized or toxic substances have been formed, growth slows down or is completely stopped.
- The biomass increases only gradually or remains constant during this stationary phase, although the composition of the cells may change.
- Due to lysis, new substrates are released which then may serve as energy sources for the slow growth of survivors.
- The various metabolites formed in the stationary phase are often of great biotechnological interest.

Death phase

- In this phase the energy reserves of the cells are exhausted.
- A straight line may be obtained when a semilogarithmic plot is made of survivors versus time, indicating that the cells are dying at an exponential rate. The length of time between the stationary phase and the death phase is dependent on the microorganism and the process used.
- The fermentation is usually interrupted at the end of the log phase or before the death phase begins.

2. Fed - batch fermentation

- In the conventional batch process just described, all of the substrate is added at the beginning of the fermentation.
- An enhancement of the closed batch process is the **fed batch** fermentation.
- In the fed-batch process, substrate is added in increments as the fermentation progresses.

- In the fed-batch method the critical elements of the nutrient solution are added in small concentrations at the beginning of the fermentation and these substances continue to be added in small doses during the production phase.

3. Continuous fermentation

In **continuous fermentation**, an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system.

- In the case of a homogeneously mixed bioreactor we refer to a **chemostat** or a **turbidistat**.
- In the chemostat in the steady state, cell growth is controlled by adjusting the concentration of one substrate.
- In the turbidistat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.

UNIT – II Media:

Media formulation – sterilization – batch and continuous sterilization, sterilization of air, fibrous filters. Microbial kinetics: batch, fed-batch and continuous cultures, phases of batch growth. kinetics of cell growth, product formation, substrate utilization, product inhibition kinetics, yield concept and productivity.

Fermenter design

The fermenter configuration and functions

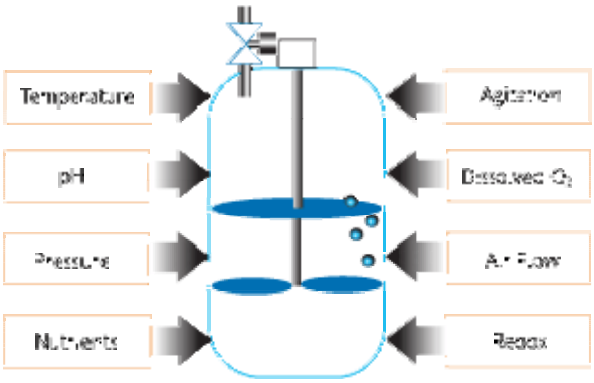
The essential configuration and general features of an ideal industrial fermenter are

1. Material used in the fabrication of a fermenter should be strong enough to withstand the interior pressure due to the fermentation medium. It should be resistant to corrosion and free from any toxic effect for the microbial culture.
2. It should provide a controlled environment for optimum biomass/product yields.
3. The nature of the construction material to be selected depends upon the particular fermentation that is to be carried out in the vessel. Fro example wooden tanks are used in carrying out some fermentations such as those producing lactic acids. On the other hand material in the fabrication of or lining of fermentation vessels include copper, stainless steel, iron and glass.
4. Stainless steel or glass is not used for lining of fermenters except in certain cases since they are costly material.
5. A fermenter should permit easy control of contaminating microbes

6. The fermentation tank should be provided with the inoculation point for aseptic transfer of inoculum.
7. In case of aerobic submerged fermentations, the tank should be equipped with aerating device.
8. A fermenter should be provided with a stirring device for the uniform distribution of air, nutrients and microbes
9. Baffles should be present to avoid vortex formation
10. The fermentation vessel should be equipped with a sampling valve for withdrawing a samples for different laboratory test.
11. There should be a provision for controlling temperature and pH of the fermentation medium.
12. There should be a provision for intermittent addition of anti-foam agents and feeding certain medium components.
13. A drain at the bottom is essential for the removal of the completed fermentation broth for further processing
14. A man hole should be provided at the top for access inside the fermenter for different purposes (repairing and cleaning).

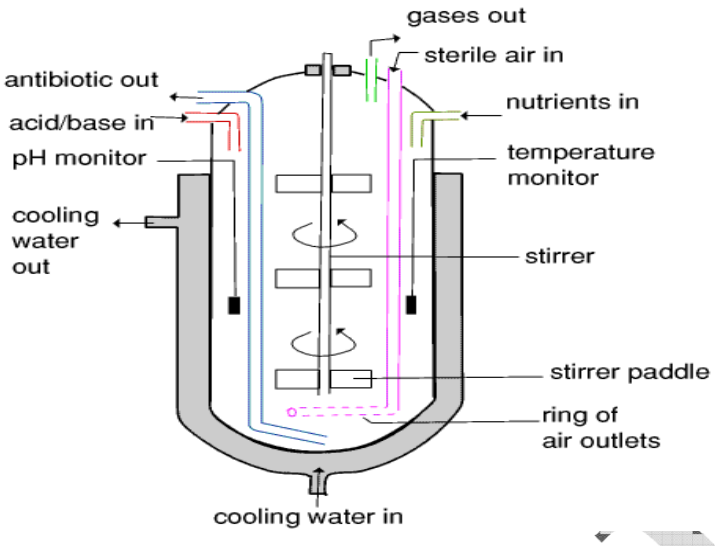
Fermenter Design and Control

- Incubation control necessitates the precise control of a number of parameters of primary importance are:



Temperature, pH, DO2 or Redox, agitation, pressure, foam control, auxiliary feed or a combination of these controllers.

- The control of these and any other parameters is most usually carried out in fermenter vessels specifically designed for the purpose and accommodating various working volumes depending on the yield and production requirements.
- Laboratory scale vessels could have a capacity of just 10 litres or less whereas production vessels may be as large as several thousand liters.
- The smallest units may incorporate an electrical heater and feed stocks (e.g. Nutrient and pH control agents) may be fed from flasks via peristaltic pumps.
- Larger vessels have an integral jacket for controlling temperature via hot or cold water and allowing indirect sterilisation using injected steam.
- Where larger quantities of feed stock are required they may be held in separate pressurized tanks and fed via a 'thrust pump' arrangement of valves.



Cross section of fermentor

- The actual fermentation process is known as the Incubation Phase and is just part of the batch cycle.
- A complete fermentation cycle can typically include the following steps (depending on vessel design):Empty (Blank) Sterilisation of vessel & pipework using direct Steam

Injection

↓

Charging with base medium

↓

Indirect Sterilisation via Steam Injected into the vessel jacket

↓

Cooling & Jacket Drain

↓

Pre-Inoculation – Vessel environment under control

↓

Inoculation – Injection of a small sample of the monoculture

↓

Incubation – The Fermentation process itself

↓

Harvesting – Product removed ready for extraction processes

- A bioreactor is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Bioreactors are extensively used for food processing, fermentation, waste treatment, etc.
- On the basis of the agent used, bioreactors are grouped into the following two broad classes: (i) those based on living cells and, (ii) those employing enzymes.
- Based on process requirements, they are of the following types: (i) aerobic, (ii) anaerobic, (iii) solid state, and (iv) immobilized cell bioreactors.
- All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other.
- Chemical engineering principles are employed for design and operation of bioreactors.
- But, in general, theoretical explanation usually lags behind technical realization. A bioreactor should provide for the following: (i) agitation (for mixing of cells and medium), (ii) aeration (aerobic fermenters, for O_2 supply), (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (iv) sterilization and maintenance of sterility, and (v) withdrawal of cells/medium (for continuous fermenters). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

Agitation and Aeration:

- In scaling up, both chemical (O_2, pH, medium constituents and removal of wastes) and physical (the configuration of bioreactor and power supplied to the reactor) factors have to be optimized for good results.
- The medium must be suitably stirred to keep the cells in suspension and to make the culture homogeneous; it becomes increasingly difficult with the scaling up.
- Various types of stirrers range from simple magnetic stirrers, flat blade turbine impellers, to marine impellers, to those using pneumatic energy, e.g., airlift fermenter, and those using hydraulic energy, e.g., medium perfusion.
- Improved mixing can be obtained by changing the design of stirrer paddle or by using multiple impellers. The objective of stirring is to achieve good mixing without causing damage to the cells.
- Vibro-mixer achieves stirring by vertical reciprocating motion of 0.1-3 mm at a frequency of 50 cycles/sec of a mixing disc fixed horizontally to the agitator shaft. These stirrers cause random mixing, less foaming and lower shear forces.
- It is important to supply sufficient O2 without damaging the cells. Mean O2 utilization rate by cells is about 6 mg $O_2/10^6$ cells/hour. But O2 is only sparingly soluble in culture medium; the oxygen transfer rate (OTR) from gas phase into medium is about 17 µg/cm/hr.
- Therefore surface aeration can support about 50 x 10^6 cells in I 1 culture vessel. Efficient aeration is achieved by bubbling air through the medium (sparging), but this may damage animal cells due to the high surface energy of the bubble and on the cell membrane
- The damage can be reduced by using larger bubbles, lower gassing rates and by adding non-nutritional supplements like Pluronic F-68 (polyglycol) and sodium carboxymcthyl cellulose (these protect cells from damage due to shear forces and bubbles, respectively).

- Silicone tubing (highly gas permeable) can be arranged inside the culture vessel (2-5 cm tubing of 30 111 length for a 1000 1 culture) and air is passed though the tube; however it is inconvenient to use.
- Aeration may be achieved by medium perfusion, in which medium is continuously taken from culture vessel, passed through an oxygenation chamber and returned to the culture.
- The cells are removed from the medium taken for perfusion so that the medium can be suitably altered, e.g., for pH control. Perfusion is used with glass bead and, more particularly, with micro-carrier systems.
- Where considered safe and desirable, O_2 supply in the culture vessel can be enhanced from the normal 21% to a higher value and the air pressure can be increased by 1 atmosphere. This increases the O_2 solubility and diffusion rates in the medium, but there is a risk of O_2 toxicity.
- The basic objective of aeration is to provide microorganisms growing in submerged cultures with adequate oxygen for their metabolic needs. Agitation, on the other hand, aims to ensure a homogeneous distribution of microorganisms and the nutrients in the broth.
- The type of aeration- agitation system used in the fermenter is dictated by the characteristics of the fermentation process. For example, in processes based on low viscosity, low total solids broths, agitation may not be needed as aeration itself would create the necessary agitation.
- Fine bubble aerators without mechanical agitation offer the advantage of lower equipment and power costs. Such fermentations are usually carried out in vessels having height/diameter ratio of 5 : 1, but a tall column of liquid would require a higher energy input for the compression of air used for aeration.
- However, mechanical agitation is usually necessary for fermentation processes based on actinomycetes and fungi. The following components of the fermenter are required for aeration

and agitation: (t) agitator (impeller), (ii) stirrer glands and bearings, (iii) baffles, and (iv) sparger (the aeration system).

1. Agitator (Impeller):

Agitators achieve the following objectives;

(a) bulk fluid and gas-phase mixing,

(b) air dispersion,

(c) oxygen transfer,

(d) heat transfer,

(e) suspension of solid particles, and

(f) maintenance of a uniform environment throughout the vessel.

- These objectives are achieved by a suitable combination of the most appropriate agitator, air sparger and baffles, and the best positions for nutrient feeds, acid or alkali for pH control and antifoam addition. Agitators are of several different types, e.g.,
- (i) disc turbines,
- (ii) vaned discs,
- (iii) open turbines of variable pitch and
- (iv) propellers.

- Disc turbine consists of a disc with a series of rectangular vanes set in a vertical plane around its perpheri. The vaned disc turbine has a series of rectangular vanes attached vertically to the underside of the disc.
- In case of variable pitch open turbine, the vanes are attached directly to a boss on the agitator shaft.
- The marine propeller is similar to variable pitch open turbine, except that it has blades in the place of vanes. In case of disc and vaned disc turbines, the air bubbles from the sparger first-hit the underside of disc before being broken into smaller bubbles and dispersed by the vanes.

- But in the case of the latter two types of agitators, air bubbles contact the vanes/blades directly and arc broken up and dispersed by them. These basic agitation devices have been variously modified. For example, the variable pitch open turbine scheme has been modified to develop four modern agitator types, viz., Scaba 6SRGT, Prochem Maxflo T, Lightning A315 and the Ekato Intcrmig.
- The Rushton disc turbine, having a diameter of one-third the fermenter diameter, has been long considered optimum for many fermentation processes. The disc turbine was considered optimum because it was shown to be able to break up a fast air stream without itself becoming flooded in air bubbles; the latter situation seriously hampers oxygen dispersal in the broth.
- In contrast, the impeller and open turbine were found to have the tendency to be flooded in air at higher aeration rates. In subsequent studies, it was found that in low viscosity broths, all the four agitator types can achieve good gas dispersion provided the agitator speed is high enough.
- In such broths, agitator type does not appear to be a significant factor affecting oxygen transfer efficiencies. In high viscosity broths, however, gas dispersal presents problems and is greatly reduced. In view of this, a number of agitators have been developed for high viscosity broths, e.g., Scaba 6SRGT, Prochem Maxflow T, Lightning A315 and Ekato Intermig.
- These agitators are larger, require lower power input (they do not lose as much power as the Rushton turbines when aerated), are able to handle higher air volumes without flooding, and give better bulk blending and heat transfer in more viscous media
- But they can cause mechanical problems mostly of vibrational nature. Good mixing and aeration in high viscosity broths may also be achieved by a dual impeller combination in which the lower impeller primarily dispenses the air, while the upper impeller primarily enhances mixing of the broth.

2. Stirrer Glands and Bearings:

- The satisfactory sealing of the stirrer shaft assembly has been one of the most difficult problems; this is very important for maintaining aseptic conditions over long periods. Four basic types of seal assembly have been used in fermenters:
- (1) the stuffing box (packed- gland seal),
- (2) the simple bush seal,
- (3) the mechanical seal and
- (4) the magnetic drive.
- Most modern fermenters use mechanical seals; these seals are more expensive, but they are more durable and less prone to leakage or contaminant entry. Magnetic drives, although quite expensive, are being used in some animal cell culture vessels
- The mechanical seal consists of two parts; one part remains stationery in the bearing housing, while the other rotates on the shaft. The two components of the seal are pressed together by springs or expanding bellows. Steam condensate is used to lubricate and cool the seals during operation and servers as a contaminant barrier.

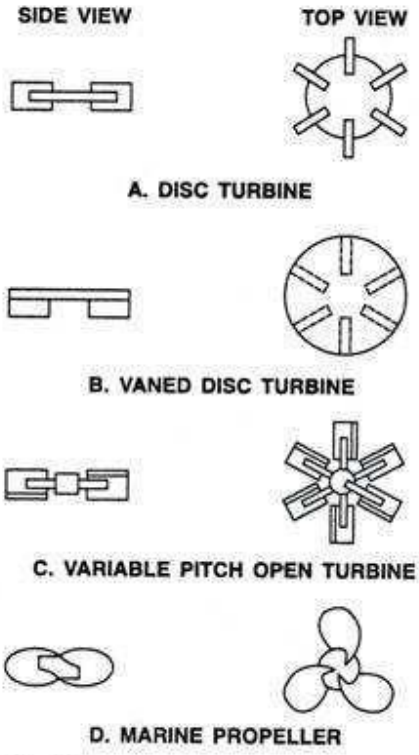
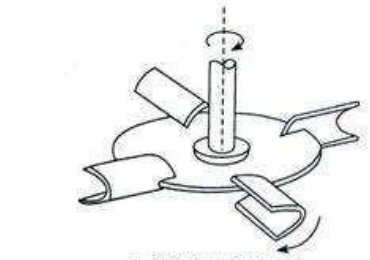
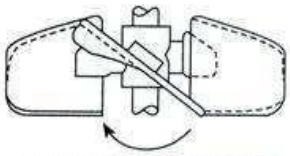


FIG. 14.1. Different types of agitators : **A.** disc turbine; **B.** vaned disc; **C.** open turbine, variable pitch; and **D.** marine propeller agitators.





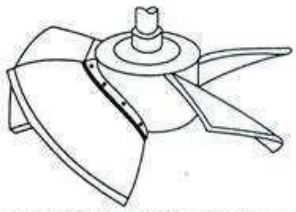


FIG. 14.2. Diagram of **A.** Scaba agitator; **B.** Lightning A315 agitator (four blades) and **C.** Prochem Maxflo T agitator (four, five or six blades).

3. Baffles:

- Baffles are metal strips roughly one-tenth of the vessel diameter and attached radially to the fermenter wall. They are normally used in fermenters having agitators to prevent vortex formation and to improve aeration efficiency.
- Usually, four baffles are used, but larger fermenters may have 6 or 8 baffles. Extra cooling coils may be attached to baffles to improve cooling. Further, the baffles may be installed in such a way that a gap exists between the baffles and the fermenter wall. This would lead to a scouring action around and behind the baffles, which would minimise microbial growth on the baffles and the fermenter wall.

4. Aeration System (Sparger):

- The device used to introduce air into the fermenter broth is called sparger. Spargers are of the following three basic types: (1) porous spargers, (2) orifice spargers and (3) nozzle spargers. Porous spargers may be made of sintered glass, ceramics or a metal.
- They are used primarily on a laboratory scale in non-agitated vessels. The bubble size from such spargers is always 10 to 100 limes larger than the pore size of the sparger. These spargers have low air throughput because pressure drops across the sparger, and the fine holes often become blocked by microbial growth.
- Orifice spargers consist of perforated pipes arranged in various ways, e.g., the sparger pipe forming a ring below the impeller. In most cases, air holes are drilled on the underside of the pipe and the holes are arranged in the form of ring or cross.
- It is desirable that the holes are at least 6 mm in diameter to avoid clogging by microbial growth. These spargers (without agitation) have been used to a limited extent in yeast manufacture, effluent treatment and in air-lift fermenters used for single-cell protein (SCP) production.

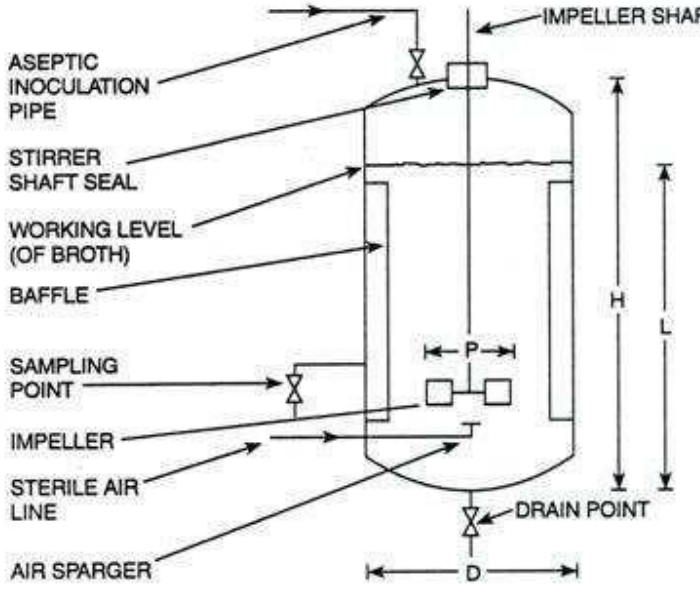


FIG. 14.3. Diagram of a fermenter with one multi-bladed impeller. H, fermenter height; L, liquid height; D, tank diameter; P, impeller diameter.

- Nozle sparger consists of an open or partially closed pipe. Most modern fermenters (laboratory to production scale) have a single open or partially closed pipe as a sparger that is ideally placed centrally below the impeller.
- It provides a stream of air bubbles. The sparger should be as far below the impeller as possible to avoid flooding of the impeller in a stream of air bubbles. These spargers cause a lower pressure loss than the other spargers and they are not easily blocked.
- In small fermenters, a combined sparger-agitator may be used. In this case, the air is introduced via a hollow agitator shaft, and it comes out through holes drilled in the disc between the blades and connected to the base of the main shaft. This design gives a good aeration in baffled vessels over a range of agitator speeds.

Temperature Regulation:

- The fermenter must have an adequate provision for temperature control. Both microbial activity and agitation will generate heat. If this heat generates a temperature that is optimum for the fermentation process, then heat removal or addition may not be required.
- But in most cases, this may not be the case; in all such cases, either additional heating or removal of the excess heat would be required. Temperature control may be considered at laboratory scale, and pilot and production scales.

1. In laboratory scale fermentations, normally little heat is generated. Therefore, heat has to be added to the system; this can be achieved in the following ways: (a) the fermenter may be placed in thermostatically controlled bath, (b) internal heating coils may be used, (c) water may be circulated through a heating jacket, or (d) a silicone healing jacket may be used. The silicone jacket consists of two silicone rubber mats, and heating wires between these mats. This jacket is wrapped around the fermenter and is held in place by Velcro strips.

2. In case of larger fermenters beyond a certain size, excess heat is generated, and the fermenter surface becomes inadequate for heat removal. The size at which fermenter surface becomes inadequate for heat

removal will depend on the fermentation process and the ambient temperature at which fermentation is being carried out. In such cases, internal coils have to be used to circulate cold water through them for removing the excess heat.

Foam Control

- Foam is produced during most microbial fermentations. Foaming may occur either due to a medium component, e.g., protein present in the medium, or due to some compound produced by the microorganism. Proteins are present in corn-steep liquor, pharma media, peanut meal, soybean meal, etc.
- These proteins may denature at the air-broth interface and form a protein film that does not rupture readily. Foaming can cause removal of cells from the medium; such cell wills undergo autolysis and release more proteins into the medium. This, in turn, will further stabilize the foam. Five different patterns of foaming are recognized; these are listed below.

1. Foaming remains at a constant level throughout the fermentation. Initial foaming is due to the medium, but later microbial activity contributes to it.

2. Foaming declines steadily in the initial stages, but remains constant thereafter. This type of foaming is due to the medium.

3. The foaming increases after a slight initial fall', in this case, microbial activity is the major cause of foaming.

4. The foaming level increases with fermentation duration; such foaming pattern is solely due to microbial activity.

5. A complex foaming pattern that combines features of two or more of the above patterns.

Foaming may lead to several physical and biological problems. Some examples of physical problems are as follows:

(1) The working volume of the fermenter may decrease due to a circulation of oxygen-depleted gas bubbles in the system.

(2) The bubble size may also decrease, and

(3) The heat and mass transfer rates may also decline.

(4) Foaming may interfere with the functioning of sensing electrodes resulting in invalid process data, and incorrect monitoring and control of pH, temperature, etc.

The biological problems of foaming include (1) deposition of cells in the upper parts of the fermenter, (2 problems of sterile operation as the air filter exits of the fermenter become wet, and (3) increased risk of contamination. In addition, (4) there may be product loss due to siphoning of the culture broth.

Whenever excessive foaming occurs, the following approaches may be used to resolve the problem:

- A defined medium may be used to avoid foam formation. This may be combined with modifications in physical parameters like pH, temperature, aeration and agitation. This approach will be successful in such cases where medium is the main culprit, but will fail whenever microbial activity is the main contributor.
- (2) Often the foam may be unavoidable; in such case, antifoam should be used. This is the most standard approach to combat foaming.
- (3) A mechanical foam breaker may also be used. Antifoams are surface active agents; they reduce surface tension in the foams and destabilize protein film by the following effects: (a) hydrophobic bridges between two surfaces, (b) displacement of the absorbed protein, and (c) rapid spreading of the surface film.

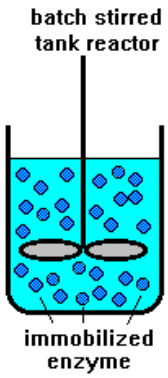
- Several compounds meet and have been found to be suitable for different fermentation processes; these compounds are as follows: alcohols (stearyl and octyl decanol), esters, fatty acids and their derivatives (especially, triglycerides like cottonseed oil, linseed oil, soybean oil, sunflower oil, etc.), silicones, sulphonates, and miscellaneous compounds like oxaline, Alkaterge C, and polypropylene glycol.

- Many of the antifoams are of low solubility; therefore, they are added with a carrier like lard oil, liquid paraffin and castor oil. There carriers, however, may be metabolized, and they may affect the fermentation process. Further, many antifoams would reduce oxygen transfer by up to 50% when used at effective concentrations.
- Antifoams are generally added when foaming occurs during fermentation. But foam control in fermentation industry is still an empirical art. Therefore, the best method of foam control for a particular process in one factory is not necessarily the best for the same process in other factories. Further, the design and operating parameters of the fermenters may affect the properties and the foams produced during the fermentation process.

Types of fermenter

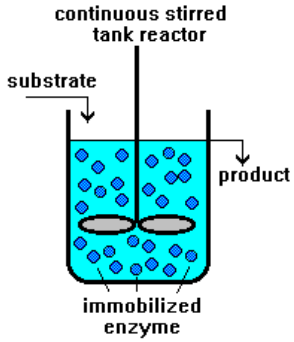
i. Stirred tank bioreactor

A **batch stirred tank** reactor is the simplest type of reactor. It is composed of a reactor and a mixer such as a stirrer, a turbine wing or a propeller. The batch stirred tank reactor is illustrated below:



This reactor is useful for substrate solutions of high viscosity and for immobilized enzymes with relatively low activity. However, a problem that arises is that an immobilized enzyme tends to decompose upon physical stirring. The batch system is generally suitable for the production of rather small amounts of chemicals.

A **continuous stirred tank** reactor is shown below:



The continuous stirred tank reactor is more efficient than a batch stirred tank reactor but the equipment is slightly more complicated.

This is the conventional mixing reactor which is made of either glass or stainless steel. The stirrer can be either at the top or bottom of the reactor. The dimentions of the reactor depend on the amount of heat to be removed from the vessel. Baffles in the centre of the tank prevent formation of vortex and effective mixing of the ingredients.

Advantages

- Low investments
- Low operation cost.

Disadvantages

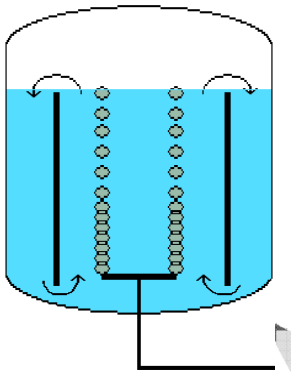
- Foaming is often a problem. But this can be overcome using proper antifoaming agents.

ii. Air lift bioreactor

Air-lift bioreactors are similar to bubble column reactors, but differ by the fact that they contain a draft tube. The draft tube is always an inner tube (this kind of air-lift bioreactor is called "air-lift bioreactor with an internal loop) or an external tube (this kind of air-lift bioreactor is called "air-lift bioreactor with an external loop) which improves

circulation and oxygen transfer and equalizes shear forces in the reactor. The figure below illustrates the basic structure of an air-lift bioreactor with an internal loop.

The stirred tank bioreactors lack well defined flow of air. In these, air is pumped from below. This creates the bubbles in the medium which rises up through the draught tube by buoyancy and drags the surrounding fluid up. The air that is used to lift up is sufficient to stir up the content.



Advantages

- Low friction
- Less energy requirement
- The mechanical parts are easy to construct. There is no need of special aseptic seals.
- Scaling up is easier
- Metabolic performance does not drastically reduce on scale up.

Disadvantage

- Capital needed is more
- Difficulty of sterilization
- Efficiency of mixing is low

Tower FermenteR

- A tower fermenter has been defined by Green-shields and co-workers as an elongated non-mechanically stirred fermenter that has an aspect ratio (height to diameter ratio) of at least 6 : 1 for the tubular section and 10 ; 1 overall, and there is a unidirectional How of gases through the fermenter.
- There are several different types of tower fermenters, which are grouped as follows on the basis of their design: (1) bubble columns, (2) vertical-tower beer fermenter and (3) multistage fermenter systems.

1. Bubble Column Tower Fermenters:

These are the simplest type of tower fermenters; they consist of glass or metal tubes into which air is introduced at the base. Fermenter volumes from 3 / to up to 950 / have been used, and the aspect ratio may be up to 16 : 1. These tower fermenters have been used for citric acid and tetracycline production, and for a range of other fermentations based on mycelial fungi.

2. Vertical-Tower Beer Fermenters:

These fermenters were designed for beer production and to maximise yeast biomass yields. A series of perforated plates are placed at intervals to maximise yeast yields. It has a settling zone free of gas; in this zone, yeast cells settle down to the bottom and return to the main body of the tower fermenter, and clear beer could be removed from the fermenter. Tower of up to 20,000 / capacity and capable of producing up to 90,000 I beer per day have been installed.

3. Multistage Tower Fermenters:

In these fermenters, a column forms the body of vessel, which is divided into compartments by placing perforated plates across the fermenter. About 10% of the horizontal area of plates is perforated. In a variant of this type of fermenter (down-flow tower fermenter), the substrate is fed in at the top and overflowed through down spouts to the next section, and the air is supplied from the base. These fermenters have been used for continuous culture of E. coli, S. cerevisiae (baker's yeast), and activated sludge.

Bubble-up Fermenter:

- It is a bubble column fermenter that is fitted with an internal cooling coil (Fig. 14.8). Air is introduced from the bottom of the column. In this vessel, the cooling coil effectively separates the column into an inner riser/draught tube and the outer down-flow tube. The cooling coil assembly functions as a leaky draught tube.
- The culture broth rises in the compartment enclosed by the cooling coils and it moves down in the compartment outside the coil, although back- mixing also occurs through the coils. The region above the cooling coil shows good mixing, and there were no poorly oxygenated zones in the vessel. It can generate liquid velocities of 1 m sec^{-1}, giving circulation times of 9-12 seconds and mixing times of 14-18 seconds.

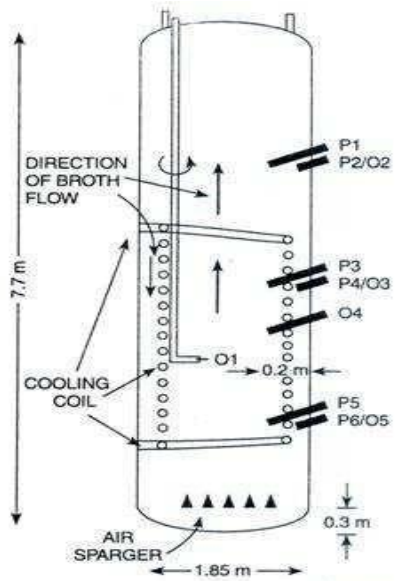


FIG. 14.8. Schematic diagram of a 20 m^3 bubble column fermenter fitted with internal cooling coil. P_1, P_2, etc. indicate location of pH electrodes; and O1, O2, etc. depict the locations of oxygen electrodes to measure the amount of dissolved oxygen.

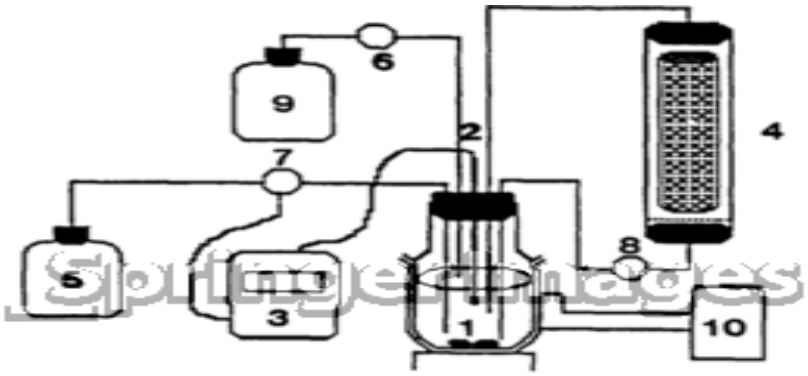
a. Anaerobic fermentation

These reactor do not require aeration except in a few where initial preparation of inoculums requires aeration. Once the fermentation starts off, the gas released from the media is sufficient to provide mixing.

In case of enzyme production, the recovery has to be strictly under anaerobic conditions since for most of the enzymatic activity is sensitive to the preseance of oxygen.

b. Immobilized cell bioreactor

- Cell immobilization is a technique to fix plant cells in a suitable matrix. Cell immobilization is different from cell entrapment in that immobilized cells can be entrapped cells but also the cells are absorbed onto support materials
- Immobilization is now a well-established technique with the history of enzyme immobilization going back over 25 years and including many industrial applications. The immobilization of microorganism is less well developed in terms of large-scale application, but it is widely used in the laboratory.
- With this background it was inevitable that immobilization techniques should be applied to plant cell cultures and much work has been carried out to establish methods for plant cell immobilization and suitable bioreactors for use with the immobilized cultures.



1. Bioreactor Reservoir
2. pH Electrode
3. pH Controller
4. Bioreactor Column with Immobilized Cells
5. Milk Feedstock
6. Outflow Pump
7. Feedstock Pump
8. Bioreactor Pump
9. Acidified Milk Product
10. Thermostated Water Bath

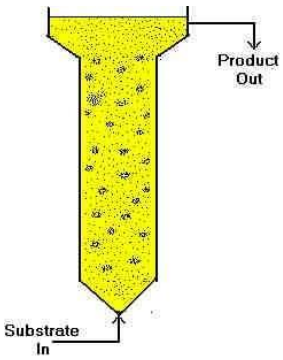
Advantages

- Useful fro manufacture of intracellular enzymes
- When the extracted enzymes are unstable
- For preparing low weight products which are released into the medium
- Reduction of pollution
- Allow continuous operation of bioreactors
- Suitable for production of amino acids, organic acids etc.,

Commonly fluidized bed reactors and hollow fiber membrane bioreactors are used

i. Fluidized bed reactors

- A **fluidized-bed reactor** is a combination of the two most common, packed-bed and stirred tank, continuous flow reactors. It is very important to chemical engineering because of its excellent heat and mass transfer characteristics. The fluidized-bed reactor can be seen below:



- In a fluidized-bed reactor, the substrate is passed upward through the immobilized enzyme bed at a high enough velocity to lift the particles. However, the velocity must not be so high that the enzymes are swept away from the reactor entirely.
- This causes some mixing, more than the piston-flow model in the packed-bed reactor, but complete mixing as in the CSTR model.
- This type of reactor is ideal for highly exothermic reactions because it eliminates local hot-spots, due to its mass and heat transfer characteristics mentioned before. It is most often applied in immobilized-enzyme catalysis where viscous, particulate substrates are to be handled.
- These reactors can utilize high density of particles and reduce bulk fluid density.

Advantages

- Heat and mass transfer are efficient
- The mixing of the media between the liquid, solid and gaseous phases are effective
- The reactor requires less energy
- Low shear rates and hence suitable for cells which are more sensitive to friction like the plant cells and mammalian cells.

ii. Hollow fiber membrane bioreactors

These reactors have hollow fibers are made from cellulose acetate, acrylic polymers, polysulphone etc.,

Advantages

- Extra cellular products can be separated from cells at the same time
- The productivity is high
- Scale up is easy since several parallel fiber units can be added

Disadvantages

- Sometimes, the pores get plugged
- Cell growth around the lumen can sometimes distort and rupture the fibers
- Nutrients and products can diffuse through the membrane and limit the growth of microbes
- If the toxic products happen to accumulate in the fiber it may inhibit the growth of microbes.

iii. Trickle Bed Reactors

- Trickle beds with countercurrent flow of gas and liquid are used on a large scale for vinegar production, as biofilters for gas clean-up and deodorisation, for water purification and for ore leaching.
- Trickle beds can be operated with or without recycling, but recycling allows higher loading and gives better flow distribution, which is even more critical than in submerged packed bed operation. Cleaning is again possible by flooding the filter and proceeding as with submerged fixed bed reactors.

- In the case of gas purification, the gas is cleaned in a single passage and the liquid is there both as an absorption fluid and as nutrient supply to the biomass on the packing (usually wood shavings or bark). Excess biomass is settled out of this stream and no other cleaning is needed.

Fermentation based on the media and substrate condition

1. Solid-State Fermentation (SSF)

2. Submerged Fermentation (SmF)/Liquid Fermentation (LF)

Solid-State Fermentation (SSF)

- Solid state fermentation involves the growth of microorganisms on moist solid particles in situation in which the spaces between the particles contains a continuous gas phase and a minimum of visible water.
- Although droplets of water may be present between the particles and there may be thin films of water at the particle surface, the inter-particle water phase is discontinuous and most of the inter-particle space is filled by the gas phase.
- The majority of the water in the system is absorbed within the moist solid – particles.
- SSF utilizes solid substrates, like bran, bagasse, and paper pulp. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates.
- In this fermentation technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for long fermentation periods.
- Hence, this technique supports controlled release of nutrients.
- SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content.

- However, it cannot be used in fermentation processes involving organisms that require high aw (water activity), such as bacteria.

Submerged Fermentation (SmF)/Liquid Fermentation (LF)

- SmF utilizes free flowing liquid substrates, such as molasses and broths.
- The bioactive compounds are secreted into the fermentation broth.
- The substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients.
- This fermentation technique is best suited for microorganisms such as bacteria that require high moisture Substrates content.
- An additional advantage of this technique is that purification of products is easier.
- SmF is primarily used in the extraction of secondary metabolites that need to be used in liquid formThe raw materials used in fermentation constitute the substrates for the fermentation process.
- The substrates could be seen as either the raw materials that will be ultimately transformed into the desired fermentation products or they could be regarded as the source of nutrients for the fermentation microorganisms.
- The substrates form the bulk of the fermentation broth and often considered as one of the most important component in the cost of the fermentation products.
- Thus in order to lower the costs of production the search for the most cheapest and economical source of fermentation substrate will be the top most agenda in any proposed fermentation industry.

- The search for the most suitable substrate is not only dictated by costs and availability of the substrate but by other factors such as complexity of unwanted reactions that affect not only upstream, midstream but downstream activities.
- This would also mean the problem of treating its effluent from polluting the environment Agro-industrial residues are generally considered the best substrates for the Solid state fermentation (SSF) processes, and use of SSF for the production of enzymes is no exception to that.
- A number of such substrates have been employed for the cultivation of microorganisms to produce host of enzymes.
- Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc.
- Wheat bran however holds the key, and has most commonly been used, in various processes.
- The selection of a substrate for enzyme production in a SSF process depends upon several factors, mainly related with cost and availability of the substrate, and thus may involve screening of several agro-industrial residues.
- In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells.

- The substrate that provides all the needed nutrients to the microorganisms growing in it should be considered as the ideal substrate.
- However, some of the nutrients may be available in sub-optimal concentrations, or even absent in the substrates. In such cases, it would become necessary to supplement them externally with these.
- It has also been a practice to pre-treat (chemically or mechanically) some of the substrates before using in SSF processes (e.g. ligno-cellulose), thereby making them more easily accessible for microbial growth.
- Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size and moisture level/water activity are the most critical.
- Generally, smaller substrate particles provide larger surface area for microbial attack and, thus, are a desirable factor.
- However, too small a substrate particle may result in substrate agumulation, which may interfere with microbial respiration/ aeration, and therefore result in poor growth.
- In contrast, larger particles provide better respiration/aeration efficiency (due to increased inter-particle space), but provide limited surface for microbial attack.
- This necessitates a compromised particle size for a particular process.

Media formulation

- Most fermentations require liquid media, often referred to as broth, although some solid-substrate fermentations are operated.
- Fermentation media must satisfy all the nutritional requirements of the microorganism

and fulfill the technical objectives of the process.

- The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite.

  Formulation process expressed as

  Carbon energy sources + Nitrogen source + O_2 + other requirements

  Biomass + Product + CO_2 + H_2O + heat

Role of Media in Fermentation

- In most industrial fermentation processes there are several stages where media are required.
- They may include several inoculum (starter culture) propagation steps, pilot-scale fermentations and the main production fermentation.
- The technical objectives of inoculum propagation and the main fermentation are often very different, which may be reflected in differ-ences in their media formulations.
- Where biomass or primary metabolites are the target product, the objective is to provide a production medium that allows optimal growth of the microorganism.
- For secondary metabolite production,. such as antibiotics, their biosyn-thesis is not growth related. Consequently, for this purpose, media are designed to provide an initial period of cell growth, followed by conditions optimized for secondary metabolite production.
- At this point the supply of one or more nutrients (carbon, phosphorus or nitrogen source) may be limited and rapid growth ceases.
- Media used in the cultivation of microorganisms must contain all elements in a form

suitable for the synthesis of cell substance and for the pro-duction of metabolic products.

- In laboratory research with microorganisms, pure defined chemicals may be used in the production of culture media, but in industrial fermentations, complex, almost undefinable (interms of composition) substrates are frequently used for economic reasons.
- Depending on the particular process, from 25 to 70% of the total cost of the fermentation may be due to the carbohydrate source.
- In many cases, media ingredients are byproducts of other industries and are extremely varied in composition.

Considerations made when formulating media for fermentation.

- An optimally balanced culture medium is mandatory for maximal production. A supplement of critical elements must be used if necessary.
- The composition of culture media must constantly- be adapted to the fermentation pro-cess. New batches of substrate have to be carefully evaluated in trial fermentations be-fore they can be used in production.
- In addition to product yield, product recovery must be examined in trial fermentations.
- If catabolite repression or phosphate repression cannot be eliminated by optimi-zation of the nutrient medium or suitable fer-mentation management (e.g. feeding), deregulated mutants must be used as production strains.
- Besides material cost and product yield, it must be considered whether materials used are readily available in sufficient supply without high transportation costs, and whether impurities will hinder product recovery or increase cost of product recovery

Media Requirements and Media formulations

- Most fermentations, except those involving solid substrates, require large quantities of water in which the
- medium is formulated.
- General media requirements in-clude a carbon source, which in virtually all industrial fermentations provides both energy and carbon units for biosynthesis, and sources of nitrogen, phosphorus and sulphur.
- Other minor and trace elements must also be supplied, and some microorganisms require added vitamins, such as biotin and riboflavin.
- Usually, media incorporate_buffers, or the pH is controlled by acid and alkali additions.
- Antifoam agents may be required.

The initial step in media formulation is the, examination of the overall process based on the stoichiometry for growth and product formation.

- This primarily involves consideration of the input of the carbon and nitrogen sources, minerals and oxygen and their conversion to cell biomass, metabolic products, carbon dioxide, water and heat.
- From this information it should be possible to calculate the minimum quantities of each element required to produce a certain quantity of biomass or metabolite.
- Typically, the main elemental formula of microbial cells is approximately C4H7O2N, which on the basis of dry weight is 48% C, 7% H, 32%O and 14% N. Ideally, a knowledge of the complete elemental composition of the specific industrial microorganism allows further media refinement.

- This ensures that no element is limiting, unless this is desired for a specific purpose.
- Once the elemental requirements of a microorganism have been established, suitable nutrient sources can be incorporated into the media.

Factor for media formulation

- Compounds that are rapidly metabolize may repress product formation. To overcome this, intermittent or maintain a relatively-low concentration that is not repressive.
- Certain media nutrients or environmental conditions may affect the physiology, biochemistry, and morphology of the microorganism.
- In some yeasts the single cells may develop into pseudo-mycelium or flocculate, and filamentous fungi may form pellets. This is not desirable as it affects the product yieldThe media adopted also depend on the scale of the fermentation.
- For small-scale laboratory fermentations pure chemical are often used in well defined media.
- However, this is not possible for most industrial – scale fermentation processes, simply due to cost, as media components may account for up to 60-80% of process expenditure.
- Industrial-scale fermentations primarily use cost-effective complex substrates, where many car-bon and nitrogen sources are almost un-definable.
- Most are derived from natural plant and animal materials, often byproducts of other industries, with varied and variable composition.
- Small-scale trials are usually performed with each new batch of substrate,

particularly to examine the impact on product yield and product recovery.

The main factors that affect the final choice of individual raw materials are as follows.

1. Cost and availability: ideally, materials should be inexpensive, and of consistent quality and year round availability.
2. Ease of handling in solid or liquid forms, along with associated transport and storage costs, e.g. requirements for temperature control.
3. Sterilization requirements and any potential de-naturation problems.
4. Formulation, mixing, complexing and viscosity characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages
5. The concentration of target product attained, its rate of formation and yield per gram of substrate utilized
6. The levels and range of impurities, and the potential for generating further undesired products during the process.
7. Overall health and safety implications

A medium that is easily sterilized with minimum thermal damage is vitally important.

- Thermal damage not only reduces the level of specific ingredients, but can also pro-duce potentially inhibitory byproducts that may also interfere with downstream processing.
- Other media characteristics can affect product recovery and purification, and the ease with which the cells are separated from the spent medium.

ENERGY SOURCES

SUBSTRATES USED AS CARBON SOURCES

- Carbohydrates are traditional energy sources in the fermentation industry.
- Pure glucose or sucrose can seldom be used as the sole carbon source, except in processes,

which demand exact fermentation control as they are very expensive media

Sugar beet and Cane molasses

- Molasses, a byproduct of sugar production, is one of the cheapest sources of carbohydrate.
- Besides a large amount of sugar, molasses contains nitrogenous substances, vitamins, and trace elements.
- How-ever, the composition of molasses varies de-pending on the raw material used for sugar production.
- Table below shows a comparison of the analysis of sugar beet and sugar cane molasses

Composition of sugar beet and sugar cane molasses

- Dry matter, Sucrose, Raffinose, Invert sugar, Miscellaneous organic materials, N, P,Os, CaO, MgO, K,O, SiO, AI,O3, Fe,O3, Ash, Thiamine, Riboflavin, Pyridoxine, Niacinamide, Pantothenic acid, Folic acid and Biotin

Malt Extract

- Malt extract, an aqueous extract of malted barley, is an excellent substrate for many fungi, yeasts, and actinomycetes.
- Dry malt extract consists of about 90-92% carbohydrates, and is composed of hexoses (glucose, fructose), disaccrides (maltose, sucrose), trisaccharides (maltotriose), and dextrins,.
- Nitrogenous substances present in malt extract include proteins, peptides, amino acid, purines, pyrimidines, and vitamins.
- The amino acids composition of different malt extracts varies according to the grain used, but praline always makes up about 50% of the total amino acids present.

- Culture media containing malt extract must be carefully sterilized. When overheating occurs, the Maillard reaction results, due to the low pH value and the high proportion of reducing sugar.
- In this conversion, the amino groups of amines, amino acid (especially lysine), or proteins react with the carbonyl groups of reducing sugars, aldehydes or ketones, which results in the formation of brown condensation products.
- These reaction products are not suitable substrates for microorganisms.
- The Maillard reaction is one of the main causes of damage to culture media during heat sterilization, resulting in considerably reduced yields

Typical composition of malt extract

Composition	(%)
Maltose	52.2
Hexoses (glucose, fructose)	19.1
Sucrose	1.8
Dextrin1	5.0
Other carbohydrates	3.8
Nitrogenous materials	4.6
Ash	1.5
Water content	2.0

Starch and dextrins can be directly metabolized as carbon sources by amylase-producing organisms. In addition to glucose syrup, which is frequently used as a fermentation substrate, starch has become more important as a substrate for ethanol fermentation.

Sulfite waste liquors, sugar-containing waste products of the paper industry, which have a dry

weight of 9-13%, are primarily used in the cultivation of yeasts. Sulfite liquors from coniferous trees have a total sugar content of 2-3%, and 80% of the sugars are hexoses (glucose, man-nose galactose), the others being pentoses (xylose arabinose). Sulfite liquors from deciduous contain mainly pentose sugars.

- Because of its wide availability and low cost, cellulose is being extensively studied as a substrate for conversion to sugar or alcohol. It is usually not possible to use cellulose directly as a carbon source, so it must first be hydrolyzed chemically or enzymatically.
- The sugar syrup formed from cellulose hydrolysis has been used for ethanol fermentation, and the fermentative production of butanol, acetone, and isopropanol is also being considered. Work is in progress to develop one-step processes for direct conversion of cellulose to ethanol, using fermentative organisms which produce cellulases.
- Whey, a byproduct of the dairy industry, is produced annually on a world-wide basis to the amount of 74 million tons (containing 1.2 million tons of lactose and 0.2 million tons of milk pro-tein). Only about 56% of this product is used for human or animal feed.
- The lactose is used primarily for the production of ethanol or single-cell protein, but also in the production of xanthan gum, vitamin B12, 2,3-butandiol, lactic acid, and gibberellic acid. Because of storage and transportation costs, whey is often not economical as a substrate.

- Animal fats such as lard and animal and plant oils are readily utilized by some microorganisms, but are generally added as supplemental substrates rather than as the sole fermentable carbon source. For instance, in certain antibiotic fermentations, soy, palm, and olive oils are used.

- With respect to its carbon content, methanol is the cheapest fermentation substrate, but it can be metabolized by only a few bacteria and yeasts.
- Methanol has commonly been used as a substrate for single cell protein production.
- Research has been carried out on processes for the production of glutamic acid, serine, and vitamin B12 using methanol as the sole carbon .source or as a co-substrate.
- Ethanol is available in ample supply from the fermentation of either saccharified starch or cel-lulose, and can be metabolized by many micro-organisms as the sole carbon source or as a co-substrate.
- Acetic acid, for instance, is presently made by the oxidation of ethanol.
- At the present time, the cost of ethanol is too high to make it utilizable as a general industrial carbon source.

Alkanes with a chain length of C12 to C18 are readily metabolized by many microorganisms.

The use of alkanes as an alternative to carbo-hydrates depends on the price of petroleum.

Other raw materials

- Maize grain
- Starch
- Cereals
- Potatoes
- Cassava
- Barley grains (malt)
- Sugar cane (molasses)

SUBSTRATES USED AS NITROGEN SOURCES

- Many large-scale processes utilize ammonium salts, urea, or gaseous ammonia as nitrogen source.
- A nitrogen source, which is efficiently metabolized, is corn steep liquor, which is formed during starch production from corn.
- The concentrated extract (about 4% nitrogen) contains numerous amino acids, such as alanine, arginine, glutamic acid, isoleucine, threonine, valine, phenylalanine, methionine, and cystine. The sugar present in corn steep liquor becomes largely converted to lactic acid (9-20%) by lactic acid bacteria.
- Yeast extracts are excellent substrates for many microorganisms. They are produced from baker's yeast through autolysis at 50-55%C through plasmolysis in the presence of high concentrations of NaCl.
- Yeast extract contains amino acids and peptides, water-soluble vitamin, and carbohydrates.
- The composition of yeast extract varies partly because the substrates used for yeast cultivation affect the quality of the yeast extract.
- Peptones (protein hydrolysates) can be utilized by many microorganisms but they are relatively expensive forindustrial application. Sources of peptones include meat, casein, gelatin, keratin, peanut seeds, soy meal, cotton seeds, and sunflower seeds.
- Peptone composition varies depending upon its origin. For instance, peptone from gelatin is rich in proline andhydroxypro-line, but has almost no sulfur-containing amino acids. On the other hand, peptone from keratin has a large proportion of proline and cystine, but lacks lysine.

- Peptones of plant origin (soy peptone, cottonseed peptone) have large proportions of carbohydrates. The end product is also influenced by the type of hydrolysis, whether acid or enzymatic, especially in regard to its tryptophan content.
- Soy meal, the residue from soybeans after the extraction of soybean oil, is a complex substrate. Analysis shows a protein content of 50%, a carbohydrate content of 30% (sucrose, stachyose-, raffinose, arabinoglucan, arabinan, and acidic polysaccharides), 1% residual fat, and 1.8% lecithin.
- Soy meal is frequently used in, antibiotic fermentations; catabolite regulation does not occur because of the slow catabolism of this complex mixture

Raw materials :

- Corn steep liquor
- Soya meal
- Peanut meal
- Cotton seed meal

Water sources

- Water is the major components of all fermentation media preparation
- When assessing the stability of water supply it is important to consider pH, dissolved salts, and effluent contaminations
- The mineral content of the water is very important is brewing and mashing process of fermentation.
- The reuse or efficient use of water normally high priority

Oils and fats

- Mainly the oil and fats used as anti-foam agents in fermentation process
- Vegetative oils will be used as oil sources i.e. olive oil, maize oil, cotton seed oil, lin seed oil, etc.,
- These oils contains fatty acids, oleic acid, linoleic acid, linolenic acid etc., these acids some time used as carbon sources.

Hydrocarbons and their derivatives

- n-alkanes – for the production of organic acid, amino acid, vitamins, nucleic acid, antibiotics, enzymes and proteins
- Methane

Minerals

- Microorganism requires certain mineral element for growth and metabolism i.e. magnesium, phosphorus, potassium, calcium, sulphur and chlorine are essential elements
- Others such as cobalt, copper, iron, manganese, molybdenum and zinc are used as elemental sources for microorganism.

STERILIZATION

Air and media sterilization

- It is processes that effectively kills or eliminate transmissible agents such as fungi, bacteria and virus from a surface of equipments, food materials, medications and biological culture medium.
- A process which removes all living things
- Therefore, sterilization is an essential requirement

Sterilizing types and agents

1. Wet heat up to 100ºC
2. Wet heat above 100ºC
3. Dry heat
4. Irradiation
5. Chemical Sterilization
6. Filtration

Wet heat above 100ºC

- In practice, nearly complete sterilization is needed
- So more severe methods are required
- Boiling point can be increased by heating water under pressure
- This need special equipment called "autoclave"
- With the knowledge of inactivation kinetics for heat resistant bacterial spores, suitable **autoclaving cycles** have been developed
- Killing action includes hydrolysis in addition to other factors.

Dry heat

Steam

- Steam should be at maximum water holding capacity, but with no water droplets
- Shouldn't be superheated/dry
- Non-condensable gases (air) in the steam can reduce the temperature at a given pressure

- With a 50% mixture of steam & gas the temperature at 15 psi will fell down to 112ºC, instead of 121ºC

Table 1 Standard autoclave cycles

Temperature (°C)	Time (min)	Pressure (bar)[a]	Survival[b]	Equivalent time[c] (min)
115	30	0.7	1 in 10^4	60
121	15	1.0	1 in 10^8	15
126	10	1.4	1 in 10^{17}	4.7
134	3	2.0	1 in 10^{32}	0.8

[a] 1 bar = 10^5 Pa.
[b] For a relatively heat-resistant bacterial endospore such as *Bacillus stearothermophilus*.
[c] Time required to give endospore survival of 1 in 10^8, i.e. time equivalent to 121 °C for 15 min.

Irradiation

a) Ultra-violet Light

- UV at 260 nm inactivates micro-organisms and viruses
- Acts on nucleic acids and cause
  - ◇ Strand breakage
  - ◇ Strand cross-linking
  - ◇ Pyrimidine dimmer formation

Uses

Generally used as a supplementary option for

- Air sterilization
- Surface sterilization

Limitations

- Intensity of UV source decreases with time
- Poor penetration
- Bacterial and fungal spores are resistant
- DNA repair system in microbes may repair limited damage

b) **Gamma Radiations**

- Direct effect on nucleic acids
- Indirect damage by production of Free Radicals
- Very deep penetrations
- 2.5 M. rad is a standard for sterilization (Obtained from Cobalt 60)

Advantages

- Deep penetration
- Packed items can be sterilized
- Heat sensitive items e.g. plastic-ware and antibiotic can be sterilized

Limitations

- Not usable at laboratory scale
- Spores and some viruses are somewhat resistant
- Some viruses are extremely resistant

Chemical Sterilization

a) Fumigation

- Formaldehyde, ethylene oxide or ethylene glycol may be used
- Effective against all microbes including viruses

- Effective at higher temperatures and at high humidity i.e. (75-100%)
- Conditions reducing access of fumes to microbes reduce activity (e.g. wrapping in organic or inorganic matter)

b) Liquid Disinfectants

Applications

- Routine hygiene & disinfection (surfaces & equipment)
- Disinfection of cell culture items after use, and before washing and re-sterilization
- Treatment of used or contaminated cell culture media before disposal

Methods of Application

a) Cloth/cotton b) Sprayers

c) Paper towels d) Immersion

	Effectiveness[a]			
Type	**Fungi**	**Bacteria**	**Endospores**	**Viruses**
Aldehydes	+	+	+	+
Hypochlorites	+	+	+	+
Phenolics	+	+	–	+/v
Alcohol	–	+	–	+/v

[a] Effective (+) and non-effective (–). In the case of non-enveloped viruses, the effect may be variable or partial (v) depending on the particular virus. Examples of

Limitations/Factors to be considered

- Range/anti-microbial activity less than some other agents as heat
- Spores/some viruses may be resistant

- Some disinfectants are neutralized by organic matter
- Stability of working dilutions varies
- Required exposure dependent on nature of the agent
- Extent of toxicity be considered

Filtration

a) Fluid Filtration

i) For Bacteria and Fungi

- Depth filters developed in late 1800's
- Un-glazed porcelain, diatomaceous earth, asbestos used
- Largely replaced by 0.2µm membranes
- Act as sieves
- Used especially for solutions which can't be subjected to autoclaving e.g. sera, proteins, growth promoting substances

Types of Filters

- Cellulose acetate, cellulose nitrate and their mixtures
- Nylons or polysulfones
- Polyvenylidene difluoride (PVDF), low protein binding
- Polycarbonate by irradiation-etch technique

Batch sterilization

- Batch sterilization is the reduction of contaminant organisms through the heating of a vessel.

- The entire volume of media is sterilized at once through the use of thermal or radiation techniques.
- When running a thermal batch sterilization, a system goes through 3 steps: heating, holding, and cooling. Heating requires the addition of energy throughout the entire medium volume.
- This can be done by adding heat through a jacket on the vessel.
- The batch sterilization is carried out in 30-60 minutes at 121°C, continuous sterilization is normally accomplished in 30-120 seconds at 140°C.
- The temperature is increased until it reaches the sterilization temperature where it is held for a set period of time. During this phase, most of the unwanted microorganisms are destroyed.
- Finally, the system is cooled to bring the sterile media back to the desired temperature.
- For radiation sterilization, the process is similar to above, although it uses radiation intensity instead of heat.

Advantages:

Most widely used technique

Simple operation

No additional materials are added to the media itself

Disadvantages:

More expensive heat requirements than continuous sterilization

Best results occur in well-mixed closed vessels

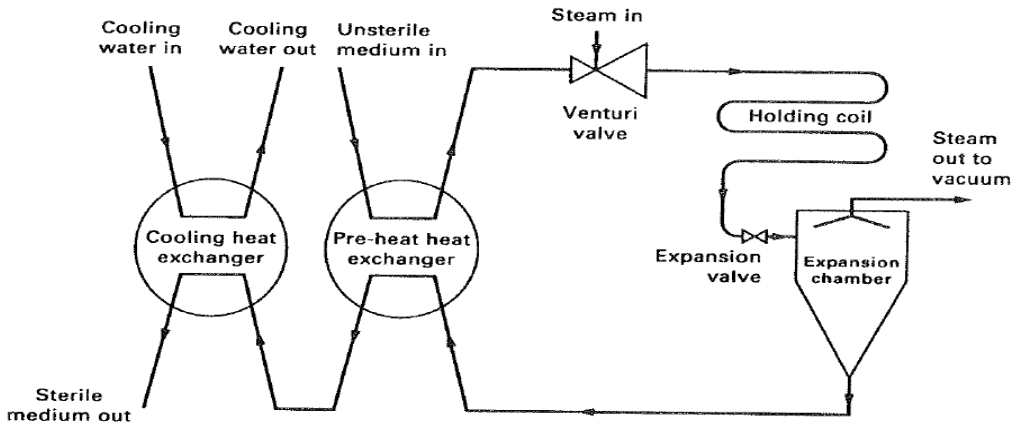
Continuous Sterilization

Batch sterilization wastes energy and can overcook the medium

- Batch sterilization uses steam or direct firing to elevate the temperature, and then cooling water stops the process and brings the material back toward room temperature.
- Both the heat and the cooling water are spent with no opportunity for energy recovery.
- Large volumes should be passed continuously through heat exchangers for energy economy with the hot, treated fluid heating the cold, incoming feed.
- One method of continuous sterilization injects steam into the medium (no heat exchanger). The medium stays in a loop for a predetermined holding time until the entire medium is sterile.
- Better heat economy comes from substituting heat exchangers for direct steam injection.
- Instead of having a cold water stream to cool the sterile media, the lower temperature unsterile media stream absorbs heat from the warm stream, cooling the sterile media.
- A system for continuous sterilization has a holding coil for detention long enough to kill all of the microorganisms.
- The medium from a make up vessel flows through the exchanger, is held in the coil, and passes back through the heat exchanger, heating more unsterile medium while becoming cool itself, as it is collected in a sterile fermenter.
- This design would work only with an exchanger with infinite heat transfer area because there is no driving force for heat transfer as the temperatures for the two streams approach closely.
- A real design would have another small exchanger to raise the temperature to the setpoint after the main exchanger has done all it can do.

- There is no need for a cooler before entering the fermenter because it has a jacket or coils for temperature control that can easily handle this load.
- Heat economy is not important for a small pilot plant unit for continuous sterilization, so direct steam injection is simpler.
- A heat exchanger is then needed with cooling water to bring the medium back down quickly to a temperature at which it is not over cooked.

There are two types of continuous sterilizer which may be used for the treatment of fermentation media: the indirect heat exchanger and the direct heat exchanger (steam injector).



Flow diagram of a typical continuous injector-flash cooler sterilizer.

Advantages:

□ Uniform steam requirements throughout the duration of the sterilization

□ Simplified process control

□ Shorter sterilization time means less thermal degradation of medium

Disadvantages:

□ High demand for steam in a shorter period of time than batch

□ Concentration of media becomes dilute due to steam condensation

□ Since steam is actually dispersed in media, steam must be clean to avoid contamination

Air Sterilization

- The magnitude of the air sterilization problem is seen from the usual needs of a highly aerobic fermentation where roughly 1 volume of air per volume of medium per minute may be used. For a factory with 20 fermenters of 100,000 L each, 2 million L/m (70,000 ft3 /m) of air is handled.
- Very large compressorsare used, and at least two are required so that one can be down for maintenance.
- In the past, air filters were columns that approached diameters of one-fourth of the fermenter diameter.
- The packing was slag wool that lumped up with repeated use, fiberglass that broke down because of repeated thermal expansion and contraction, or beads of carbon that sometime underwent spontaneous combustion and melted the column. Carbon packing works fairly well but is too bulky.
- Currently, there is a pronounced trend to use of membrane filters in a cartridge configuration for air sterilization to obtain excellent performance
- with units of relatively small size.
- Moisture is bad for all methods of air sterilization and may help microorganisms to pass. A membrane pore size of 0.2 to 0.3 micrometers is recommended.
- Hydrophillic membranes should not be used because moisture held tightly in the pores is not dislodged unless there is quite high pressure drop across the membrane. Moisture tends to drain from hydrophobic membranes and collect in a sump.

- The units are modular and housed in a shell with a manifold. Sizing is based on the number of cartridges needed.
- Air leaving a vessel in which pathogenic organisms are cultured is sterilized by heating. Air in a room for culturing microorganisms may be exposed to ultraviolet light to reduce the number of potential contaminants.
- Ultraviolet light penetrates poorly through glass, so organisms in shake flasks are not killed. Usually, a single light switch turns on white light before a person enters, and the u.v. goes on when the person flips the switch on leaving.
- There are also ultraviolet lights mounted in flow devices for water sterilization, but quartz bulbs or enclosures are needed to circumvent the attenuation of u.v. wavelengths. Such devices are also plagued by turbidity in the water and by dirt forming on the transparent surfaces.
- There have been some attempts to commercialize enzymatic sterilization of air. The basic concept is to bring microorganisms and viruses into contact with enzymes that attack nucleic acids. Viruses are destroyed by passage through a labyrinth of surfaces coated with deoxyribonuclease enzymes.

Filters are usually classified as:

- Fibrous Filters
- Porous Membrane Filters
- Capillary Porous Membrane Filters
- Fabric Filters

Fibrous Filters

- Fibrous filters consist of a mat of fine fibers arranged in such a way that most are perpendicular to the direction of air flow. It is mostly air inside.
- Thus the porosity is high -- from 70% to more than 99%. The size of the fibers ranges from submicrometers to 100 mm.
- The most common types of fibers in fibrous filters are cellulose (wood) fibers, glass fibers, and plastic fibers. The air velocity inside fibrous filter is often in the order of 10 cm/s.

Porous Filters

- The porosity is less, ranging from 50% to 90%. Due to the complex pore structure, the gas inside follows an irregular path.
- These filters have high efficiency and greater pressure drop than other types of filters. Porous filters include cellulose esters, sintered metals, polyvinyl chloride, Teflon, and other plastics.

Capillary Porous Membrane Filters

- This type of filter has a matrix of cylindrical pores of uniform diameter and approximately perpendicular to the filter surface.
- These filters are particularly used for collecting particles in a scanning electron microscope because of their smooth surface.

Fabric Filters

- These types of filters are often confused with fibrous filters. They are especially useful in industrial cleaning of high dust concentrations.
- They usually consist of large parallel fabric bags, woven or felted. Their efficiency depends upon the dust layer that is built up on the fabrics.

- The porous dust layer supported by the fabric is what makes the filter efficient. A simplified example is a home vacuum cleaner.

UNIT – III Design of fermenter:

Types – CSTR, Tower, jet loop, air lift fermenter, bubble column, packed bed. Fundamentals of process control and monitoring – on line and off line analysis, feedback control, PID controller, computer aided control.

Fundamentals of process control and monitoring

- The success of a fermentation depends upon the existence of defined environmental conditions for biomass and product formation. To achieve this goal it is important to understand what is happening to a fermentation process and how to control it to obtain optimal operating conditions.
- Thus, temperature, pH, degree of agitation, oxygen concentration in the medium and other factors may have to be kept constant during the process.
- The provision of such conditions requires careful monitoring (data acquisition and analysis) of the fermentation so that any deviation from the specified optimum might be corrected by a control system. Criteria which are monitored frequently are listed along with the control processes with which they are associated.

There are three main classes of sensor:

1. In-line sensor. The sensor is an integrated part of the fermentation equipment and the measured value obtained from it is used directly for process control.

2. On-line sensor. Although the sensor is an integral part of the fermentation equipment, the measured value cannot be used directly for control. An operator must enter measured values into the control system if the data is to be used in process control.

3. Off-line sensor. The sensor is not part of the fermentation equipment. The measured value cannot be used directly for process control. An operator is needed for the actual measurement (e.g, medium analysis or dry weight sample) and for entering the measured values into the control system for process control.

Temperature

- The temperature in a vessel or pipe is one of the most important parameters to monitor and control in any process. It may be measured by mercury-in-glass thermometers, bimetallic thermometers, pressure bulb thermometers, thermocouples, metal-resistance thermometers or thermistors.
- Metal-resistance thermometers and thermistors are used in most fermentation applications. Accurate mercury-in-glass thermometers are used to check and calibrate the other forms temperature sensors, while cheaper thermometers still used with laboratory fermenters.

Mercury-In-Glass Thermometers

- A mercury-in-glass thermometer may be used directly in small bench fermenters, but its fragility stricts its use. In larger fermenters it would be necessary to insert it into a thermometer pocket in to vessel, which introduces a time lag in registering the vessel temperature.
- This type of thermometer can used solely for indication, not for automatic control recording.

Electrical resistance thermometers

- It is well known that the electrical resistance metals changes with temperature variation
- This property has been utilized in the design of resistant thermometers. The bulb of the instrument contains to resistance element, a mica framework (for very accurate measurement) or a ceramic framework (robust; but for less accurate measurement) around which the sensing element is wound.

- A platinum wire of 100Ω resistance is normally used. Leads emerging from the bulb are connected to the measuring element.
- The reading is normally obtained by the use of a Wheat stone bridge circuit and is a measure of the average temperature of the sensing element.
- This type of thermometer does have a greater accuracy (± 0.25%) than some of the other measuring devices and is more sensitive to small temperature changes. There is a fast response to detectable changes (1 to 10 seconds), and there is no restriction on distance between the very compact sensing point (30 X 5 mm) and the display point of reproducible readings.
- These thermometers are normally enclosed in stainless-steel sheaths if they are to be used in large vessels and ancillary equipment.

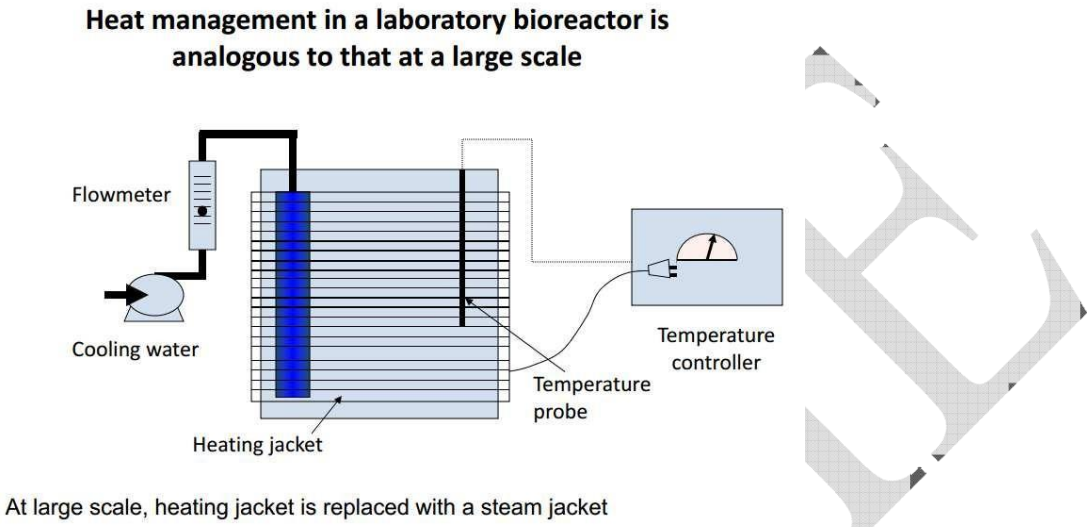
Thermistors

- Thermistors are semiconductors made from specific mixtures' of pure oxides of iron, nickel and other metals. Their main characteristic is a large change in resistance with a small temperature change.
- The change in resistance is a function of absolute temperature. The temperature reading is obtained with a Wheatstone bridge or a simpler or more complex circuit depending on the 'application.
- Thermistors are relatively cheap and have proved to be very stable, give reproducible readings, and can be sited remotely from the read out.

Temperature control

- The use of water jackets or pipe coils within a vessel the temperature has been controlled In many small systems there is heating element, 300 to 400 W capacity being adjusted. The heating element should be as well as possible to reduce the size of the 'heat sink' resulting overshoot when heating is no longer required.

- In some cases it may be better to run the cooling water continuously at a steady rate and to have heating element only connected to the control unit. This can be an expensive mode of operation if the flows directly to waste.



pH control and monitoring

- Most bacteria can grow over a wide range of pH, although many enzymes upon which microbial growth depends function only within a narrower range of pH.
- The bacteria then must maintain their internal pH near a fixed optimal value.
- Bacteria (E. coli) that grow at neutral pH (6.0 Bacteria (E. coli) that grow at neutral pH (6.0-8.0) are called neutrophiles. 8.0) are called neutrophiles
- .Regardless of the external pH, the internal pH is maintained at ~7.6.pH is maintained by ion pumps on the membrane of the bacteria.
- Effort put into maintaining the pH will be at the expense of other cellular functions Bugs tend to grow more slowly when the pH is not at the optimum.Overfeeding substrate can cause the cells to produce organic acids, such as acetate – pH dropsLack of carbohydrate substrate causes the cells to consume protein in the media – producing NH3 → NH4OH – pH rises

- When producing a protein product, cells consume ammonia from the media from the cellular demand for more nitrogen – causes the release of a proton – pH drops.
- The main purpose of a pH sensor is to maintain a pH level during fermentation that can optimize the catalytic conversion to alcohol. A classic pH meter measures the acidity or alkalinity of a liquid.
- The pH meter involves the application of glass electrodes. Any pH meter will need to be calibrated before being used to test for the acid/alkalinity of a test solution. The pH meter does require regular calibration so that the glass electrode generates an accurate pH reading.
- A standard calibration process should be carried out with two buffer solutions that cover a spectrum of pH values. It is acceptable to use buffer solutions at a pH value of 4 and 10.
- It is important to set the pH meter to two control pH values that corresponds to the two pH buffer values. The pH meter is also adjusted to a control value for temperature. By calibrating the pH meter for all three control values, the meter can achieve linear accuracy.
- A standard pH meter involves glass electrodes and reference electrodes (such as Ag/AgCl [silver/silver chloride]) that are important for measuring the pH of a test liquid. Electrical circuits containing a glass electrode, a reference electrode, and an instrument to measure electrical potential between opposing electrical fields are the basic units to a pH meter.
- The main purpose of the glass electrode is to carry an electrical current via a wire that submerges into the test liquid. The wire and liquid are enclosed by a thin glass tube. It is the glass electrode that becomes the vital process in measuring the PH of a solution. The membrane is made up of oxygen atoms that carry an odd cluster of electrons and this is what creates a negative electrical potential.
- During a standard testing procedure, this electrode is exposed to an acidic solution which forces the positive ions from the acid solution to bind to the glass electrode. Whilst submerged, the

electrodes need to maintain a neutral solution and so the electrons from the inner surface of the electrode move to the outer solution, but this will change the electrical potential of the testing solution.

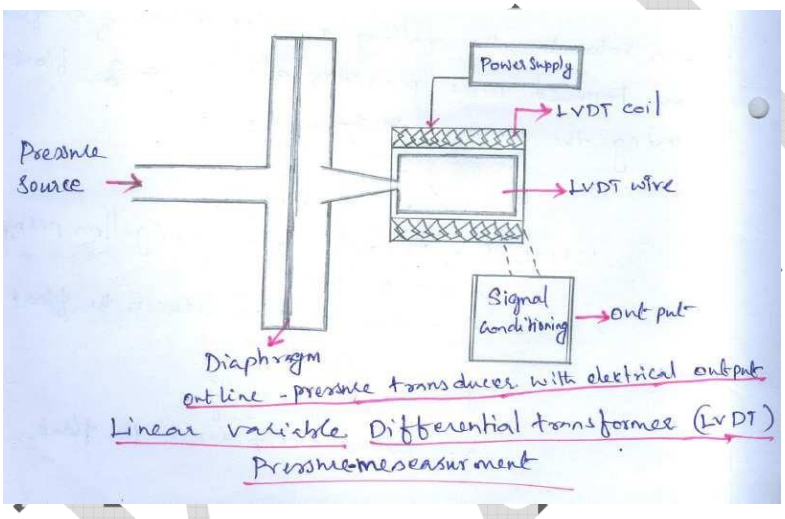
- The purpose of the reference electrode is to carry the electrical current to a meter that can measure the difference in electrical potential.
- A graduated syringe is normally used to inject dilute sodium hydroxide (neutralizer) to the wine solution together with phenolphthalein. The indicator used changes the colour of the fermented media to a pink colour to ensure that the solution is no longer acidic.
- This is where the pH meter becomes useful – this device can work to provide a neutral end point on a digital monitor.
- **In Situ Transmissive pH Sensors**
- This type of pH sensor uses sol-gel solution combined with a colorimetric pH indicator dye which can reflect light through a read fibre that will then provide an estimation of colour change to a sample solution at a certain wavelength. pH sensors are typically susceptible to changes in salinity, though the In Situ Transmissive pH Sensor is built to avoid this problem.
- This type of pH sensor works well with organic solvents including acetone, alcohols, and aromatic and so would be of benefit to the beverage brewing industry.
- **Non-Intrusive Reflective pH Sensors**

These sensors are novel and have evolved pH sensor technology. The sensor uses an electroformed mesh material which adds a coating of metal on a non-metallic surface of the pH sensor and this creates a reflective ion permeable membrane allowing for pH measurement through a clear wall to a cavity containing reflective probes. Again, this type of sensor is applicable to the food and beverage processing industry.

Pressure and control

Different working pressures are required in different parts of a fermentation plant. During normal operation a positive head pressure of atmospheres (161 kN-l) absolute is maintained in a fermenter to assist in the maintenance of aseptic conditions. This pressure will obviously be raised during a steam-sterilization cycle. The correct pressure in different components should be maintained by regulatory valves controlled by associated pressure gauges.

Pressure measurements may be made for several reasons, that most important of which is safety. Industrial and lab equipments is designed to withstand a specific working pressure a factor of safety.



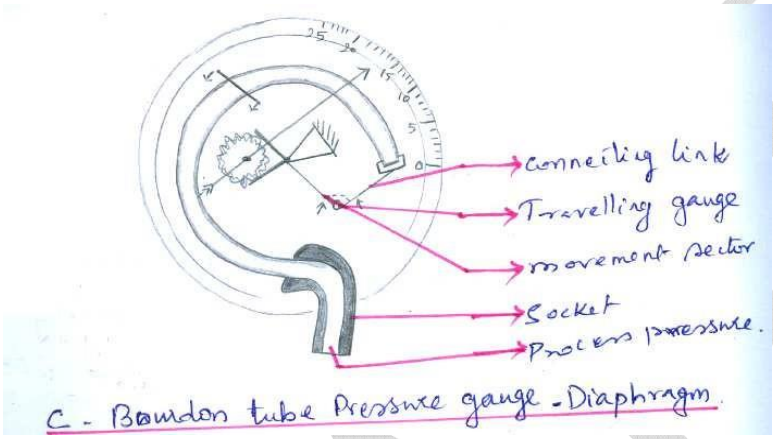
There fore the pressure indicating devices must fir in the equipments as a device. This device may be worked as sense indicate, record and control the pressure.

The measurement of pressure is important when the media sterilization and metabolic reactions in fermentation

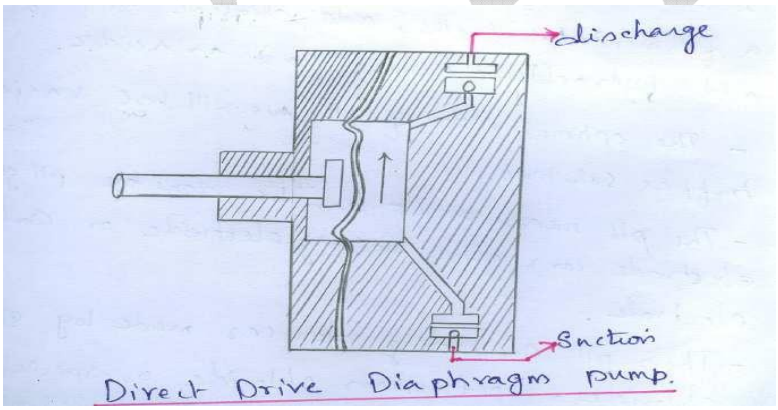
The high pressure will influence the solubility of gas and contribute to the maintenance of sterility.

This pressure can be measured by using C bourdon tube pressure gauge and it also contain direct indicating gauge.

This pressure will be maintained and controlled by using electrical output (linear variable differential transformer –LVDT). The LVDT containing primary and secondary coil, this coil movement a voltage change proportional to the displacement caused in the input pressure.



Te pressure range of 3-15 to 0-3000 psi unit . The input pressure we can controlled using direct drive diaphragm pump.



On line analysis

During fermentation, the chemical factors can influence growth and product formation and this to be continuously monitored the following techniques.

Ion specific sensor

Using this sensor to measure NH_4^+, Ca^{2+}, K^+, Mg^{2+}, PO^{3-}, So^{2-} ions concentration in the composition of the sample or fermented media.

Enzyme and microbial electrodes

Enzyme or microbial cells electrodes can be used in some analysis. A suitable enzyme or microbial cell which produces a change in pH or forms oxygen in the enzyme reaction is chosen and immobilized on a membrane held in close contact to a pH or oxygen electrode.

Enzyme electrodes are also commercially available to monitor cholesterol, triglycerides, lactate, acetate, oxalate, methanol, ethanol, creatinine, ammonia, urea, amino acids, carbohydrates, and penicillin. This electrode inserted into a fermenter and check the contents and also this type of electrode are non- thermo-stable so before inserted to the fermenter it stand with chloroform.

Near infra red spectroscopy

During fermentation the estimate fat (in media), techoic acid (in biomass) and antibiotics were analyzed and find out their NIR absorbance band with rage of 460 – 1200 nm.

Mass spectrophotometer

Mass spectrometer used to monitor the gas particle pressure of O_2, CO_2, CH_4, and dissolved gases O_2, CO_2, CH_4 and volatiles are methanol, ethanol, acetone were analysed.

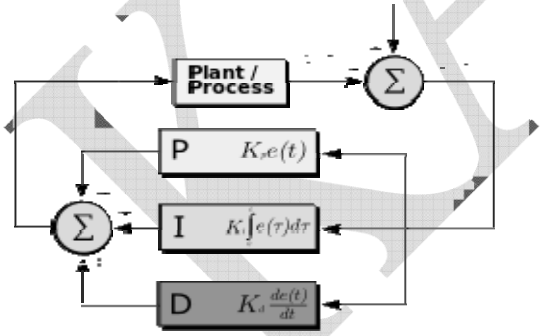
Off line analysis

Out of the fermenter performing all the analysis are called off line analysis. The analysis are pH, Temp, solubility, substrate measures, product formation check, product purity check and biomass.

PID controller,

The bioprocess control has different goals and objectives, function of bioprocess characteristics and imposed performances. In spite of high non-linearity linear control theory and basic controllers (on/off, PID) are still applied in most industrial applications.

- A proportional-integral-derivative controller (PID controller) is a generic control loop feedback mechanism (controller) widely used in industrial control systems.
- A PID controller calculates an "error" value as the difference between a measured process variable and a desired setpoint. The controller attempts to minimize the error by adjusting the process control inputs.
- The PID controller algorithm involves three separate constant parameters, and is accordingly sometimes called three-term control: the proportional, the integral and derivative values, denoted P, I, and D. Simply put, these values can be interpreted in terms of time: P depends on the present error, I on the accumulation of past errors, and D is a prediction of future errors, based on current rate of change.



Computer in bioprocess

- The bioprocess advancement is determined by the living cells capabilities and characteristics, the bioreactor performance as well as by the cultivation media composition and the main parameters evolution.

- The high metabolic network complexity inside the cells often determine very sophisticated, non-linear growth and product formation kinetics, with further consequences on the bioprocess behavior, but at the same time on the product quality and yield.
- The key issue of this rather complicated situation is the use of modeling and further on of computer assisted control as a powerful tool for bioprocess improving.
- The process models, as relationships of the input, output and inner variables, though incomplete and simplified, can be effective to describe the phenomena and the influences of great importance for control, optimization and better theoretical knowledge.
- The function of any biological model is to describe the metabolic reactions rates and their stoichiometry on the basis of bioreactor conditions, with the main difficulties-the identification of principal factors affecting cellular growth and bioproduct formation, and the building up of a suitable model structure for the intracellular processes.

Temperature

- determination is important for bioprocess evolution as well as other process operations (i.e. sterilization, concentration, and purification).
- The temperature measurement is made in the range $+20^{o}C$ to $+130^{o}C$ through mercury-in-glass thermometers, bimetallic thermometers, pressure bulb thermometers, thermocouples, metal-resistance thermometers or thermistors; all of them must be steam-sterilizable at $120^{o}C$. The most popular are the Pt100 resistance thermometers.

Pressure

- measurements may be needed for several reasons; the most important of them is the safety. Industrial and laboratory equipment is designed to withstand a specified working pressure plus a factor of safety.

- Also, the measurement of pressure is important in media sterilization.
- Moreover, the pressure will influence the solubility of gases and contribute to the maintenance of sterility, when a positive pressure is present. The standard measuring sensor is the membrane pressure gauge based on strain or capacitance measurements.
- The **formation of foam** can create serious problems in no controlled situations: loss of broth, clogging of gas analyzers, infections, etc.
- It is a common practice to add an antifoam agent when the culture starts foaming above a certain predetermined level. A standard foam sensing consists in an electrical conductivity / capacitance / heat conductivity probe.
- A number of mechanical antifoam devices have been made, including discs, propellers, brushes attached to the agitator shaft above the surface of the broth. Unfortunately, most of the mechanical devices have to be used in conjunction with an antifoam agent, without negative influence on the bioprocess behavior.

Computer in bioprocess control system.

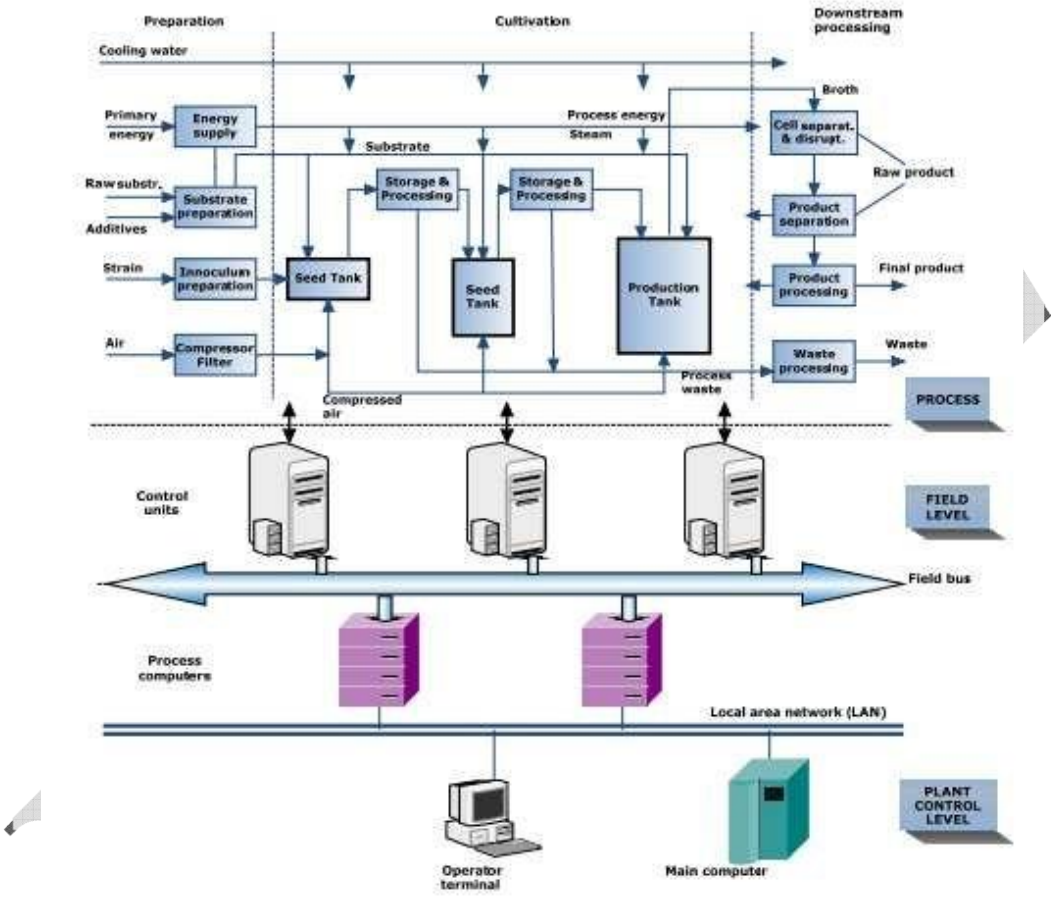
The computer aided fermentation control contains three distinct areas of computer function, that included as

- Logging of process data
- Data analysis
- Process control analysis

Logging of process data:

- Data logging is performed by the data acquisition system which has both hardware and software components.
- There is an interface between the sensor and the computer.

- The software should include the computer programme for sequencial scanning of the sensor signals and the procedure of data storage.



Data analysis

- Data reduction is performed by the data analysis system which is a computer programe based on a series of selected mathematical equation.
- The analysis information may be put on a print out, fed into a data bank or utilized for process control.

Process control

- Process control is also performed using a computer programme. Signal from the computer are fed in to pumps , valves or switches via the interface.
- In addition the computer programme may contain instructions to display devices to include alarm.
- At this poits it is necessary to be aware that there are two distinct fundamental approaches to computer control of fermentor.
- The two distinct fundamental approaches are

Direct digital control (DDC)

Fermentor is under the direct control of the computer software

Supervisory set point control (SSC)

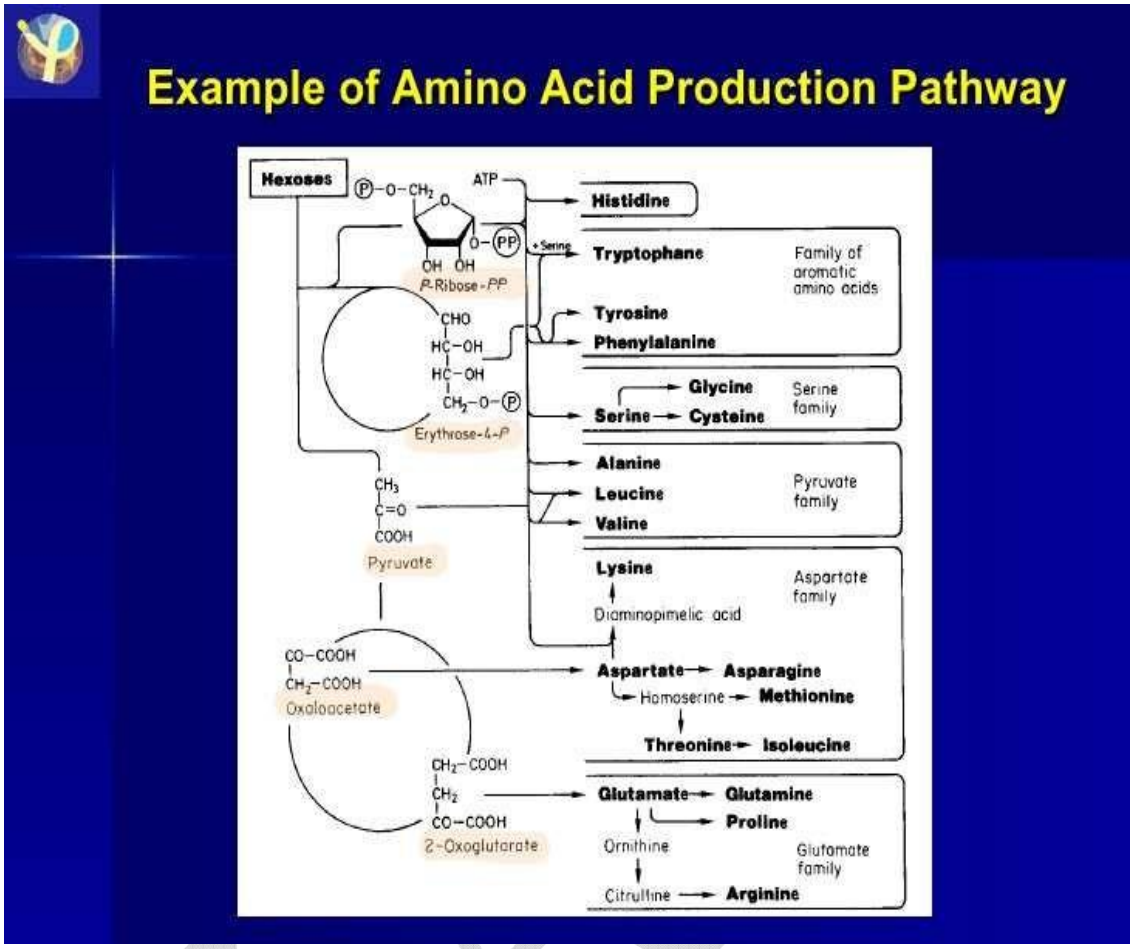
- It involves the use of independent controller to manage all control functions of a fermentor and the computer communications with the controller only to exchange information.
- All the controlling unit of fermenter such as temperature controller, pH, pressure, impeller speed, gas flow rates, liquid flow, dissolved O_2 and Co_2 controlling units with their sensor connected with software operation.
- After installation of the software an computer we can controlled all the controlling parameter of the instrument and fermentation process.

UNIT – IV Kinetics:

Transport phenomena – Rheological properties, determination of O2 mass transfer, heat transfer, role of aeration and agitation, factors affecting O2 transfer. Production of chemicals – alcohol, antibiotics – Penicillin and Streptomycin, Single cell proteins.

Production of Aminoacids

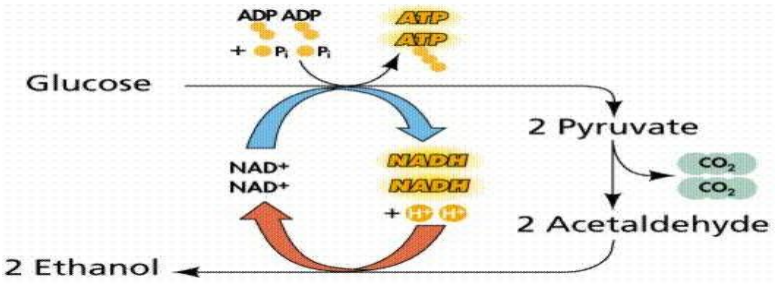
- Amino acids are the basic building blocks for proteins and nutritionally impor-tant key compounds. They have a wide variety of characteristics concerning nu-tritional value, taste, medicinal action, and chemical properties, and thus have many potential uses.
- With the exploitation of new uses, e. g., food additives, phar-maceuticals, feed supplements, cosmetics, polymer materials, and agriculturalchemicals, demand for amino acids has grown rapidly accompanied by devel-opment of mass production technology for each type of amino acid.
- The annualworld production of amino acid has increased year by year, and is currently es-timated at 2 million metric tons. The major changes in production in 1957, 1985,and 1996. At present, the amino acid industry has come to oc-cupy an important role in world chemical industries



Production of alcohol

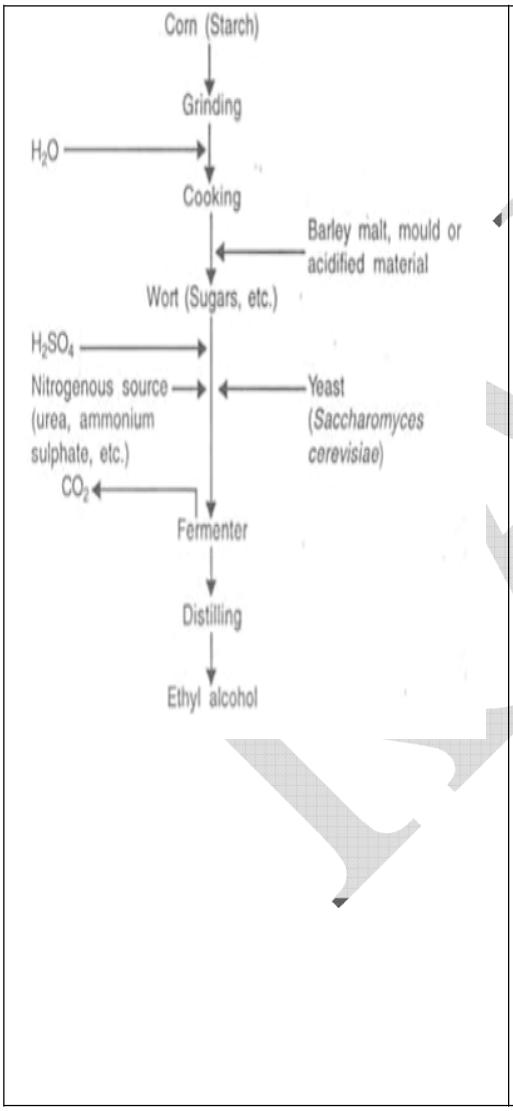
Alcohol production

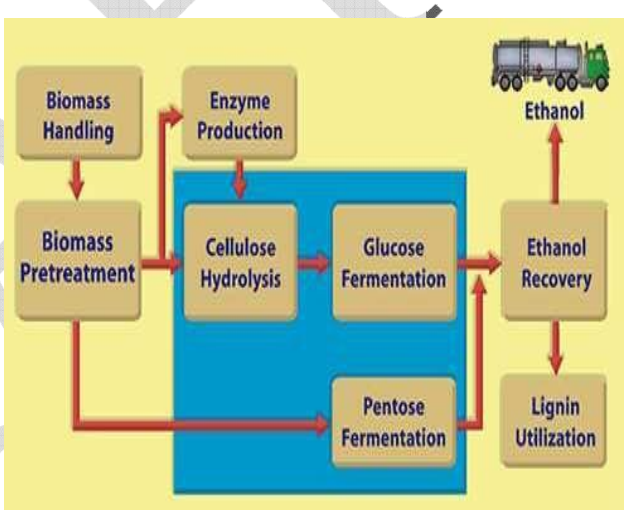
- Production of ethyl alcohol from sugary materials is one of the oldest known microbiological processes. Alcohol is an important solvent and raw material used in a variety of chemical industries.
- Although today industrial alcohol is also produced synthetically from ethylene, production of alcohol by fermentation of cheap sugary materials such as molasses by yeast is still an important industry.



- For ethyl alcohol production, selected strains of Saccharomyces cerevisae are employed since all the strains are not equally efficient.
- The alcohol tolerance and sugar tolerance are important criteria used in the selection of yeast strains. Strains tolerant to high sugar and alcohol concentration are desired.
- The raw material generally used is either crude cane molasses or best molasses which contain about 50 per cent fermentable sugars.
- The production process involves the dilution of molasses to a suitable sugar concentration(15- 16 per cent sugars), addition of small quantity of nitrogen source (urea, ammonium sulphate or ammonium phosphate), adjustment of pH to about 5.0 and the addition of anactively growing yeast culture.
- The fermentation is carried out in big deep tanks of steel or stainless steel. The fermentation is allowed to continue for about 24 – 36 h at 25^0 C to 30^0 C after which the cells are allowed to settle.
- The fermented mash is then distilled and passed through rectifying columns to recover ethyl alcohol. A large amount of carbon-di oxide is also produced during the fermentation which is purified and compressed. The yield of ethyl alcohol is about 50 per cent of the fermentable sugar concentration.

- Further purification of ethyl alcohol is done by fractional distillation. In some distilleries, the yeast is recovered and used as animal feed while in most, it is discarded into the effluents, a procedure that is very undesirable.
- In recent years because of the possibility of using ethyl alcohol as a fuel supplement and a chemical feed stock, there is increased interest in increasing production but at a cheaper and economical rate.





- For this, a variety of improvements in the traditional batch fermentation have been described in literature.
- Among these, the one that has attracted attention is the cell recycle technique which does not involve much additional expenditure. Basically, the technique involves the reuse of cell mass that is produced during the fermentation.
- It has been found that by doing so, about 5-10 per cent of the substrate which would have been otherwise used for cell growth is saved in addition to a great saving in the cost of inoculum and time.
- By using recycling technology, fermentation time has been drastically reduced from 24-36 hours in a batch fermentation to as low as 5-6 hours.

Production of antibiotics : penicillin

Production of penicillin

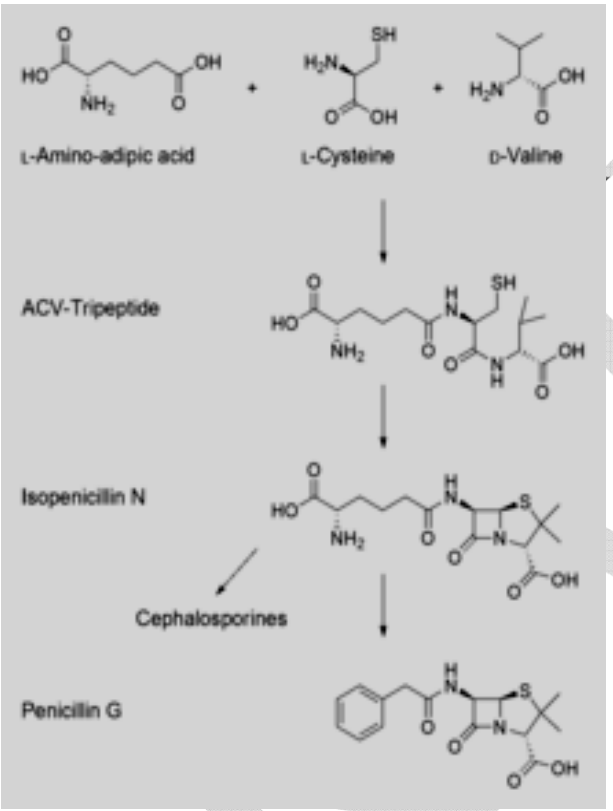
- Penicillin (sometimes abbreviated PCN or pen) is a group of antibiotics derived from *Penicillium* fungi, including penicillin G (intravenous use), penicillin V (oral use), procaine penicillin, and benzathine penicillin (intramuscular use).
- Antibiotics such as penicillin are usually produced in large cylindrical vats, constructed of stainless steel, containing a liquid medium in which *Penicillium chrysogenum* is grown.

Penicillin biosynthesis in cell

- Overall, there are three main and important steps to the biosynthesis of penicillin G(benzylpenicillin).
- The first step is the condensation of three amino acids—L-α-aminoadipic acid, L-cysteine, L-valine into a tripeptide. Before condensing into the tripeptide, the amino

acid L-valine must undergo epimerization to become D-valine. The condensed tripeptide is named δ-(L-α-aminoadipyl)-L-cysteine-D-valine (ACV).

- The condensation reaction and epimerization are both catalyzed by the enzyme δ-(L-α-aminoadipyl)-L-cysteine-D-valine synthetase (ACVS), anonribosomal peptide synthetase or NRPS.



- The second step in the biosynthesis of penicillin G is the oxidative conversion of linear ACV into the bicyclic intermediate isopenicillin N by isopenicillin N synthase(IPNS), which is encoded by the gene *pcbC*. Isopenicillin N is a very weak intermediate, because it does not show strong antibiotic activity.

- The final step is a transamidation by isopenicillin N N-acyltransferase, in which the α-aminoadipyl side-chain of isopenicillin N is removed and exchanged for aphenylacetyl side-chain.
- This reaction is encoded by the gene *penDE*, which is unique in the process of obtaining penicillins.

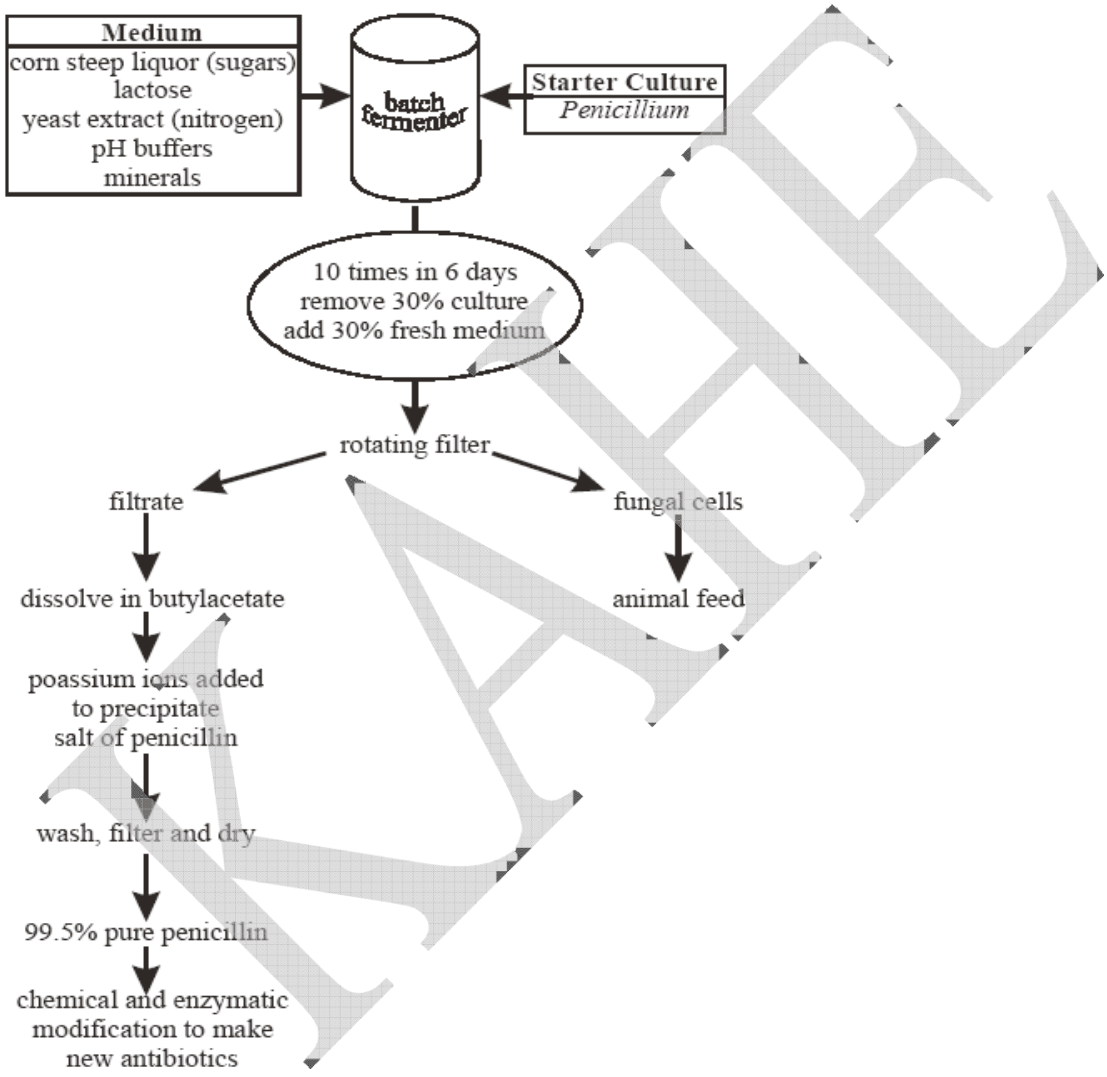
Production

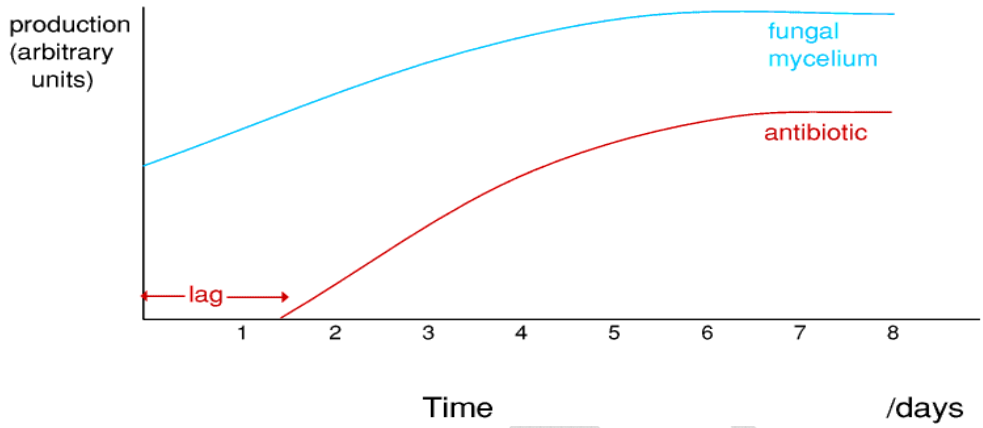
- Penicillin is a secondary metabolite of certain species of *Penicillium* and is produced when growth of the fungus is inhibited by stress.
- It is not produced during active growth. Production is also limited by feedback in the synthesis pathway of penicillin.

α-ketoglutarate + AcCoA → homocitrate → L-α-aminoadipic acid → L-lysine + β-lactam

- The by-product, L-lysine, inhibits the production of homocitrate, so the presence of exogenous lysine should be avoided in penicillin production.
- The *Penicillium* cells are grown using a technique called fed-batch culture, in which the cells are constantly subject to stress, which is required for induction of penicillin production.
- The available carbon sources are also important: Glucose inhibits penicillin production, whereas lactose does not. The pH and the levels of nitrogen, lysine, phosphate, and oxygen of the batches must also be carefully controlled.
- The biotechnological method of directed evolution has been applied to produce by mutation a large number of *Penicillium* strains.

- These techniques include error-prone PCR, DNA shuffling, ITCHY, and strand-overlap PCR.
- Semisynthetic penicillins are prepared starting from the penicillin nucleus 6-APA. Before use, fermenters must be **sterilised, usually** with **superheated steam**.





- Usually these fermenters are operated in a **batch process.** After a certain amount of time for fungal growth, followed by gradual production of antibiotic, the contents are removed and processed to extract the antibiotics, then the fermenter is cleaned, sterilised and the process is repeated.

Penicillin extraction

- After 6-8 days of batch culture, the liquid medium is pumped out, filtered and concentrated. The basic antibiotic - benzyl penicillin - is precipitated as crystals when potassium compounds are added.
- This antibiotic may then be modified by the action of other micro-organisms or by chemical means, before being mixed with inert substances and pressed into tablets or converted into syrup or injectable form.
- Although the molecular structure of penicillin is known, and it may be synthesised by chemical methods, it is not economic to do so.
- The production process still relies on fungal fermentation based on biological principles, although **modern strain**s are much **more productive** than the early strains.

- This has been achieved through screening programmes involving isolates from different sources, and treatment to encourage mutations.

Activity and Purity screening

- Using agar well and disc diffusion methods the purified streptomycin were screened their potentials with inhibiting the bacterial culture.

Production of streptomycin

- Streptomycin is a water-soluble aminoglycoside derived from Streptomyces griseus. It is marketed as the sulfate salt of streptomycin.
- Streptomycin was first isolated on October 19, 1943 by Albert Schatz, a graduate student, in the laboratory of Selman Abraham Waksman at Rutgers University.

Uses

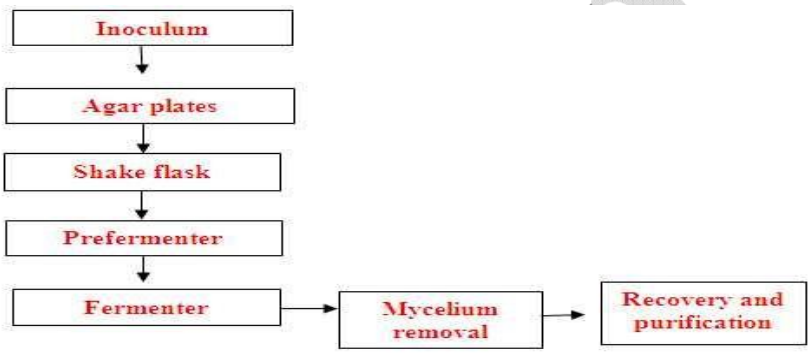
- Streptomycin can be used for the treatment of pneumonia, spinal meningitis, and typhoid fever.
- Streptomycin Sulphate Injection This product is used in the treatment of acute infections caused by various kinds of sensitive bacteria strains, like respiratory tract infections (pneumonia, laryngopharyngitis and bronchitis), urinary tract infections. Streptomycin is also used as a pesticide, to combat the growth of bacteria, fungi, and algae. A major use is in the control of fire blight on apple and pear trees.

Culturing

Isolated strain of *S. griseus* was sub-cultured on Starch casein agar slant.

Production of streptomycin

- Starch casein broth medium was used as suitable medium for the production of streptomycin.
- Prepared starch casein broth medium by adding starch, peptone beef extract, casein (1% solution) in distilled water, casein solution pasteurized at 72ºC for 30 min., When medium is prepared then inoculate the *Streptomyces griseus* into the broth. Then fermentation broth incubated in the shaker incubator for 7-8 days at 37 ºC.



Purification of the streptomycin

- A general laboratory method was used for the purification of streptomycin. In this method Centrifuged the fermentation media for 10 min. at 6000rpm and 4ºC, Collect supernatant and filtered by using wattmann`s no.1 filter paper, Taken filtrate, added 2 present (2g. in 100 ml) activated charcoal kept the solution in the shaker incubator for 20 min, Filter the solution by using wattmann's no.1 filter paper, After 2 min. added 10 ml of phosphate buffer in the residue, Taken filtrate and allow to it for crystallization at room temperature, After crystallization added 2-3 ml of phosphate buffer for dissolving the crystal.

Activity and Purity screening

- Using agar well and disc diffusion methods the purified streptomycin were screened their potentials with inhibiting the bacterial culture.

Production SCP

- Single-cell protein (SCP) typically refers to sources of mixed protein extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria (grown on agricultural wastes) used as a substitute for protein-rich foods, in human and animal feeds.

Production Process

- Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta).
- The problem with extracting single-cell proteins from the wastes is the dilution and cost.
- They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes.
- The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage.
- The methods to increase the concentrations to adequate levels and the de-watering process require equipment that is expensive and not always suitable for small-scale operations.
- It is economically prudent to feed the product locally and soon after it is produced.

Seed culture

Aspergillus niger used as seed culture to produce SCP.

The process of SCP production from any microorganism or substrate would have the following basic steps:

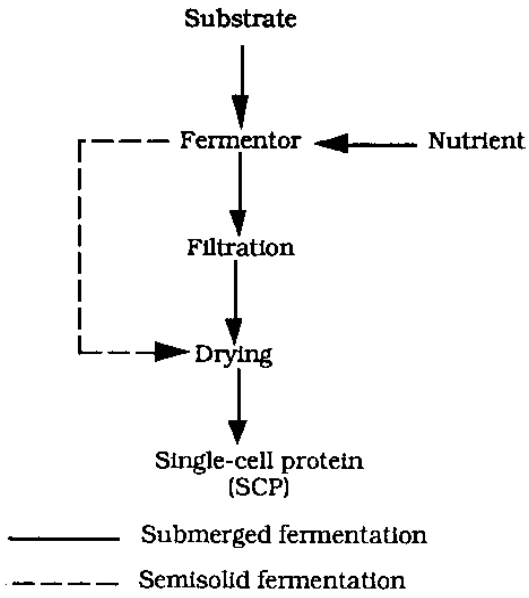
1. Provision of a carbon source; it may need physical and/or chemical pretreatments.
2. Addition, to the carbon source, of sources of nitrogen, phosphorus and other nutrients needed to support optimal growth of the selected microorganism.
3. Prevention of contamination by maintaining sterile or hygienic conditions. The medium components may be heated or sterilized by filtration and fermentation equipments may be sterilized.
4. The selected microorganism is inoculated in a pure state.
5. SCP processes are highly aerobic (except those using algae). Therefore, adequate aeration must be provided. In addition, cooling is necessary as considerable heat is generated.
6. The microbial biomass is recovered from the medium.
7. Processing of the biomass for enhancing its usefulness and/or storability.

The selection of certain microbial strain is very important, some of the criteria are:

1. Performance (growth rate, productivity, yield) on the specific. preferably low-cost substrates to be used
2. Temperature and pH tolerance
3. Oxygen requirements, heat generation during fermentation and foaming characteristics
4. Growth morphology and genetic stability in the fermentation
5. Ease of recovery, and requirements for further downstream processing
6. Structure and composition of the final product, in terms of protein

- It has been calculated that 100 lbs of yeast will produce 250 tons of proteins in 24 hours, whereas a 1000 lbs steer will synthesize only 1 lb of protein 24 hours and this after consuming 12 to 20 lbs of plant proteins.
- Similar, algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year.
- Single cell protein can be produced by two types of fermentation processes, namely submerged fermentation and semisolid state fermentation.
- In the submerged process, the substrate to be fermented is always in a liquid which contains the nutrient needed for growth.
- The substrate is held in the fermentor which is operated continuously while the product biomass is continuously harvested.
- The product is filtered or centrifuged and then dried. For semisolid fermentation, the preparation of the substrate is not as elaborate; it is also more conducive to a solid substrate such as cassava waste.
- Submerged culture fermentations are more capital intensive and have a higher operating cost when compared with semisolid fermentations which, however, have a lower protein yield.

The major proportion of the production cost in most fermentation processes is the cost of the raw materials which can be up to 25-70 percent.



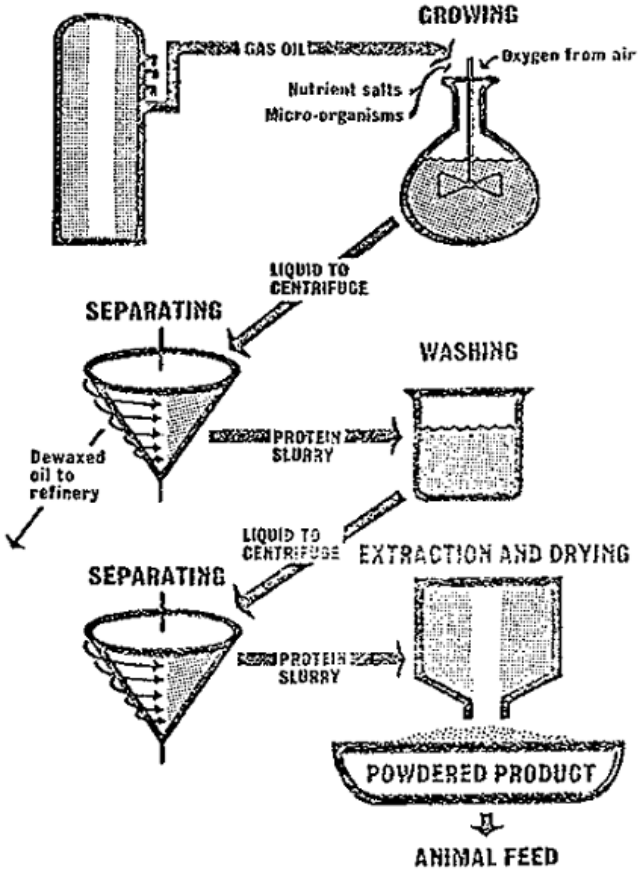
- However, the main problem of SCP production is the relatively high cost in the downstream processing and marketing SCP as food.
- Using waste materials as substrate provides lower yield of biomass than using more defined substrates, many processes are required to increase the biomass concentrations including centrifugation, flotation, precipitation, coagulation and filtration, or the use of semi-permeable membranes.
- SCP also treated with other processes to kill the microbes, increase the digestibility, and to reduce the nucleic acid content.
- These processes require extra costs which may exceed the costs for conventional food production.
- The removal or reduction of nucleic acid content of various SCP's is achieved with one of the following treatments:

a. chemical treatment with NaOH;

b. treatment of cells with 10% NaCl;

c. thermal shock.

These methods aim to reduce the RNA content from about 7% to 1% which is considered within acceptable levels.



Single Cell Protein - Yeast

- Yeast is another source of Single Cell Protein, and have been produced since a long time ago.
- In World War I, Torula yeast (*Candida utilis*) was produced in Germany and used in soups and sausages. Nowadays, the pet food industry is a major outlet of microbial biomass.

- The dog, cat. fish feed is supplemented with yeasts, it will make the product more palatable to the animals.
- Use of yeast as food seasoning is commonly found in vegetarians diet, Torula yeast has been commercially used for this purpose, an example of this product is Hickory Smoked Dried Torula Yeast.
- Yeast has some advantages among other SCP sources, such as:

1. Easy to harvest because of their size (larger than bacteria)
2. High level of malic acid content
3. High lysine content
4. Can grow at acidic pH
5. Long history of traditional use

This nutritious microbe unfortunately has few disadvantages that have to be taken as consideration, such as:

1. Lower growth rates compared to bacteria
2. Lower protein content than bacteria (45-65%)
3. Lower methionine content than bacteria, solved by the addition of methionine in the final product.

Single Cell Protein - Algae

- Since a decade ago studies on Single Cell Protein (SCP) had drawn the attention of scientist to bridge the protein gap.
- The use of algae as food and feed is known since centuries as they form part of the diets is East Asian countries as well as the natives in Central Africa.

- Some of the algae like *Chloralla*, *Soenedesmus*, *Coelastrum* and *Spirulina* have been found to suitable for mass cultivation and utilization.
- The advantages in using algae include simple cultivation, effective utilization of solar energy, faster growth and high protein and nutrient content.

Cultivation of *Spirulina and production*

In tropical countries *Spirulina* cultured under authotrophic, heterotrphic and mixotrophic conditions.

- Mass cultivation easier than other algal cultivation because aeration of CO_2 is not necessary for this species since it can maximally utilizes the amounts of carbon that are supplied by using bicarbonate of the culture medium.
- Continues agitation is not very essential to boost up the yield.
- Because the studies proofing that continuous mixing with paddle wheels or pumps and sporadic manual stirring with brooms are not providing any considerable differences in yields.
- The optimum temperature for *Spirulina* is the temperatures in between 25 and 35°C.
- The growth will be retarded when the temperatures below 20°C.
- Although *Spirulina* seems to be a more thermophilic alga, it could not withstand longer periods at temperatures above 40°C.
- Light intensities of 30-40 klux were found to be optimal for *Spirulina.*
- To maintain these intensities during summer months, scaffoldings were arranged around the ponds and roofed over sparsely with coconut fronds, which cut down the light by 40-50%, but left enough space for ventilation.

Immobilization of enzymes and microbial cells

- An **immobilized enzyme** is an enzyme attached to an inert, insoluble material—such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride).
- This can provide increased resistance to changes in conditions such as pH or temperature. It also lets enzymes be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalysed reactions.
- An alternative to enzyme immobilization is whole cell immobilization.

Commercial use

Immobilized enzymes are very important for commercial uses as they possess many benefits to the expenses and processes of the reaction of which include:

- **Convenience**: Minuscule amounts of protein dissolve in the reaction, so workup can be much easier. Upon completion, reaction mixtures typically contain only solvent and reaction products.
- **Economy**: The immobilized enzyme is easily removed from the reaction making it easy to recycle the biocatalyst. This is particularly useful in processes such as the production of Lactose Free Milk, as the milk can be drained from a container leaving the enzyme (Lactase) inside ready for the next batch.
- **Stability**: Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme.
- In the past, biological washing powders and detergents contained many proteases and lipases that broke down dirt. However, when the cleaning products contacted human skin,

they created allergic reactions. This is why immobilization of enzymes are important, not just economically.

Immobilization of an Enzyme

There are various ways by which one can immobilize an enzyme:

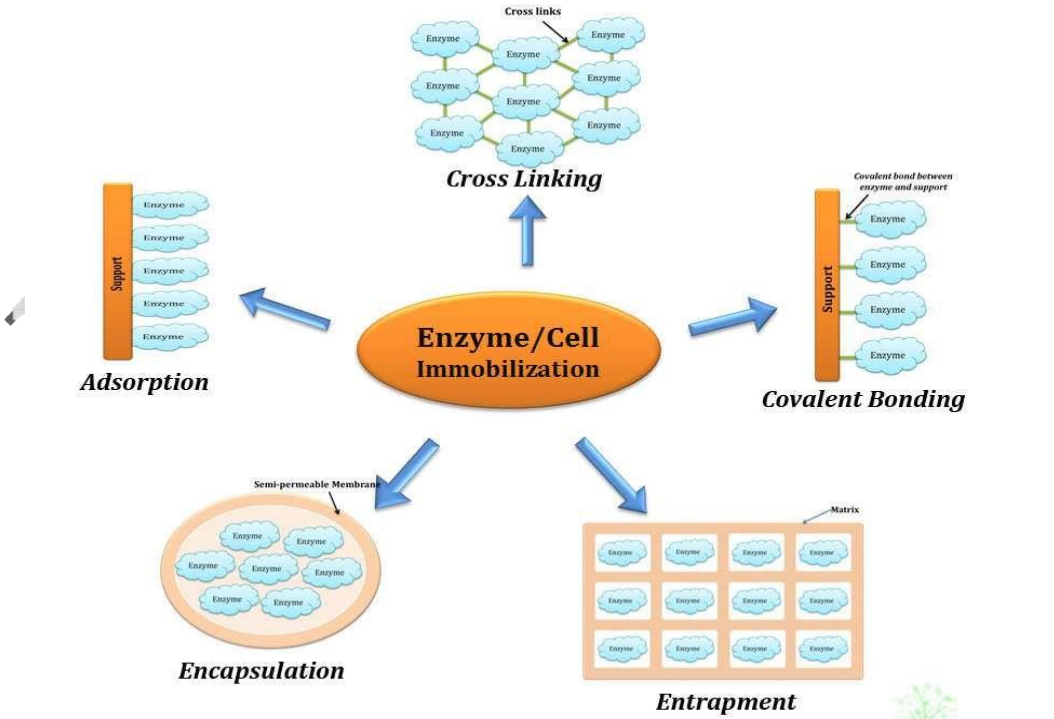
- Affinity-tag binding: Enzymes may be immobilized to a surface, e.g. in a porous material, using non-covalent or covalent Protein tags. This technology has been established for protein purification purposes, and has recently been applied for biocatalysis applications by EziG™ with the His-tag. This technique is the only one generally applicable, and can be performed without prior enzyme purification with a pure preparation as the result. Porous glass and derivatives thereof are used, where the porous surface can be adapted in terms of hydrophobicity to suit the enzyme in question.
- Adsorption on glass, alginate beads or matrix: Enzyme is attached to the outside of an inert material. In general, this method is the slowest among those listed here. As adsorption is not a chemical reaction, the active site of the immobilized enzyme may be blocked by the matrix or bead, greatly reducing the activity of the enzyme.
- Entrapment: The enzyme is trapped in insoluble beads or microspheres, such as calcium alginate beads. However, this insoluble substances hinders the arrival of the substrate, and the exit of products.
- Cross-linkage: Enzyme molecules are covalently bonded to each other to create a matrix consisting of almost only enzyme. The reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility. However, the inflexibility of the covalent bonds precludes the self-healing properties

exhibited by chemoadsorbed self-assembled monolayers. Use of a spacer molecule like poly(ethylene glycol)helps reduce the steric hindrance by the substrate in this case.

- Covalent bond: The enzyme is bound covalentely to an insoluble support (such as silica gel). This approach provides the strongest enzyme/support interaction, and so the lowest protein leakage during catalysis.

Immobilization of a Substrate for Enzymatic Reactions

- Another widely used application of the immobilization approach together with enzymes has been the enzymatic reactions on immobilized substrates.
- This approach facilitates the analysis of enzyme activities and mimics the performance of enzymes on e.g. cell wall



Methods of Cell Immobilization

The reactions catalyzed by immobilized whole-cell biocatalysts can be classified as follows:

1. Reactions involving single enzymes (bioconversions)

2. Reactions involving multienzyme systems with or without cofactors

3. Reactions involving a complete metabolic pathway yielding primary or secondary metabolites

The use of immobilized microbial cells is advantageous in the following areas

1. When the desired enzymes are intracellular and the extracted, purified enzymes become unstable after immobilization

2. When the microorganism does not contain interfering enzymes or when such enzymes can be inactivated without loss of desired catalytic activity

3. When substrates and products do not have a high molecular mass and can diffuse through the cell membrane

Immobilization material

1. The material should be available in sufficient quantities and at low price.

2. The material should have a large surface area accessible to cells and reactants

3. The material must be mechanically, chemically, and thermally stable under process and storage conditions.

4. The matrix should contain a sufficient number of functional groups to bind the cells.

5. The material should not reduce cell activity or initiate cell lysis.

6. The material should be easy to handle in the immobilization procedure.

7. The material should be capable of recycling or safe disposal.

8. In the case of viable growing cells, the matrix should have a sufficiently large void volume or be elastic enough to accommodate new cells.

Techniques

1. Carrier-free immobilization

2. Immobilization of a given biomass onto a preformed carrier surface

3. Immobilization of a given biomass during the course of carrier formation (e.g., by polymerization)

4. Immobilization by controlled growth of an inoculum or by germination of immobilized spores.

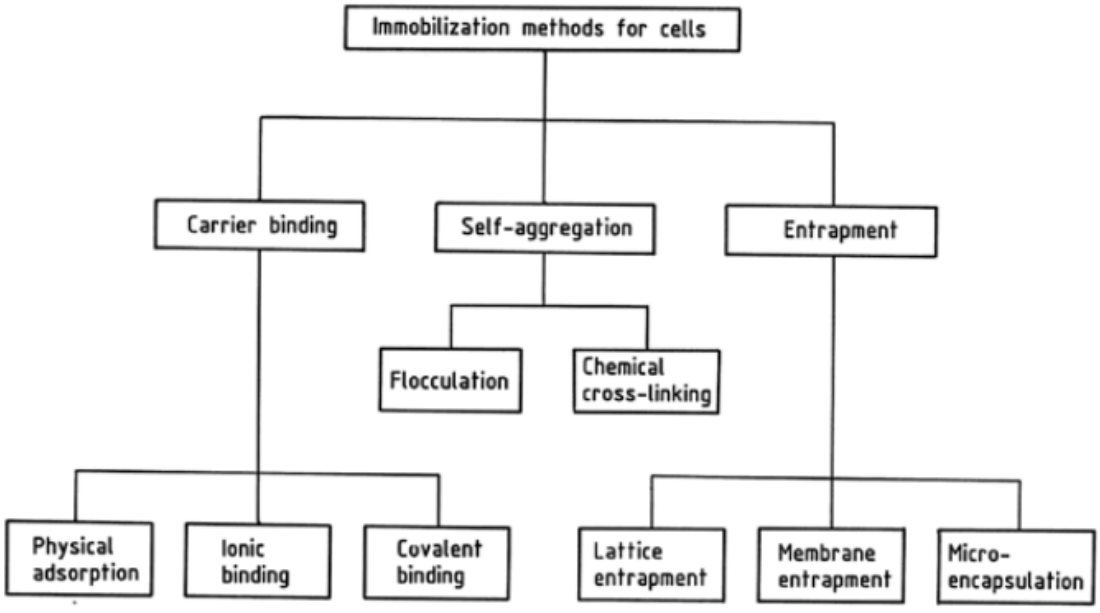


Figure.10. Types of cell immobilization

Cell Immobilization without a Support

- Intrinsic tendency to aggregate or flocculate at high cell densities. Yeast, mold, and plant cells aggregand are relatively stable to shear fields in fluidized-bed reactors.

- Flocculation can also be induced by polyelectrolytes such as chitosan. Cell aggregation can be induced by low molecular mass bi- or multifunctional reagents such as glutaraldehyde, diazotized diamines, or toluene diisocyanate.

Binding of Cells to a Carrier

Physical Adsorption

- Additional chemicals are usually unnecessary, and fixation is carried out under growth conditions; viable cell preparations can, therefore, be obtained.
- Mammalian cells bound to preformed surfaces to produce therapeutic biochemicals.
- The cells are immobilized on microcarriers (small-diameter beads, 100 – 200 µm) manufactured from different synthetic polymers (e.g., polystyrene, gelatin, dextran, polyacrylamide, or glass) that offer a large specific surface area for cell growth monolithic ceramic matrix has been developed for large-scale cell cultures.
- For adherent cell growth, the scalability is almost linear and depends on the available surface area.

Ionic Binding

- Ionic binding is a special case of physical adsorption where charged microbial cells can electrostatically interact with the ions on a carrier surface to form stable complexes.
- Synthetic ionexchange resins, modified cellulose derivatives, or inorganic materials can be used as carriers.
- Cell adsorption is mainly affected by factors such as pH, ionic strength, surface charge, cell age, or composition of the carrier surface.

UNIT -V Downstream processing:

Cell distribution methods for intracellular products; foam separation, precipitation. Filtration – micro and ultra-filtration; Solvent extraction-, chromatographic separation- FPLC, HPLC, dialysis, centrifugation, distillation, drying, crystallization, turbidity analysis and cell yield determination. Fermentation products – available in market.

- **Downstream processing** refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste.
- It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and human growth hormone), antibodies and vaccines; antibodies and enzymes used in diagnostics; industrial enzymes; and natural fragrance and flavor compounds.
- Downstream processing is usually considered a specialized field in biochemical engineering, itself a specialization within chemical engineering, though many of the key technologies were developed by chemists and biologists for laboratory-scale separation of biological products.
- Downstream processing and analytical bio-separation both refer to the separation or purification of biological products, but at different scales of operation and for different purposes.
- Downstream processing implies manufacture of a purified product fit for a specific use, generally in marketable quantities, while analytical bio-separation refers to purification for the sole purpose of measuring a component or components of a mixture, and may deal with sample sizes as small as a single cell.

Steps in downs stream process

- Removal of insolubles is the first step and involves the capture of the product as a solute in a particulate-free liquid, for example the separation of cells, cell debris or other particulate matter from fermentation broth containing an antibiotic.
- Typical operations to achieve this are filtration, centrifugation, sedimentation, precipitation, flocculation, electro-precipitation, and gravity settling.
- Additional operations such as grinding, homogenization, or leaching, required to recover products from solid sources such as plant and animal tissues, are usually included in this group.
- Product isolation is the removal of those components whose properties vary markedly from that of the desired product.
- For most products, water is the chief impurity and isolation steps are designed to remove most of it, reducing the volume of material to be handled and concentrating the product.
- Solvent extraction, adsorption, ultrafiltration, and precipitation are some of the unit operations involved.
- Product purification is done to separate those contaminants that resemble the product very closely in physical and chemical properties.
- Consequently steps in this stage are expensive to carry out and require sensitive and sophisticated equipment.
- This stage contributes a significant fraction of the entire downstream processing expenditure.
- Examples of operations include affinity, size exclusion, reversed phase chromatography, crystallization and fractional precipitation.
- Product polishing describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilization and spray drying are typical unit operations.

- Depending on the product and its intended use, polishing may also include operations to sterilize the product and remove or deactivate trace contaminants which might compromise product safety. Such operations might include the removal of viruses or depyrogenation.
- A few product recovery methods may be considered to combine two or more stages.

Cell distribution methods for intracellular products

- The importance of microorganisms as a source of commercially useful chemicals, antibiotics and enzymes has been recognized for a very long time.
- Nearly all chemicals of microbial origin produced industrially today are of the extracellular type. That is, they are produced within the microbial cell, but are then excreted into the surrounding environment.
- A much larger proportion of the potentially useful microbial products is retained within the cells. A vast majority of the enzymes known, for example, are intracellular.
- Even greater use of microbial products, many of which will be intracellular, can be expected from the predicted surge in biotechnology.
- The isolation of intracellular material requires that the cell either be genetically engineered so that what would normally be an intracellular product is excreted into the environment, or it must be disintegrated by physical, chemical, or enzymatic means to release its contents into the surrounding medium.

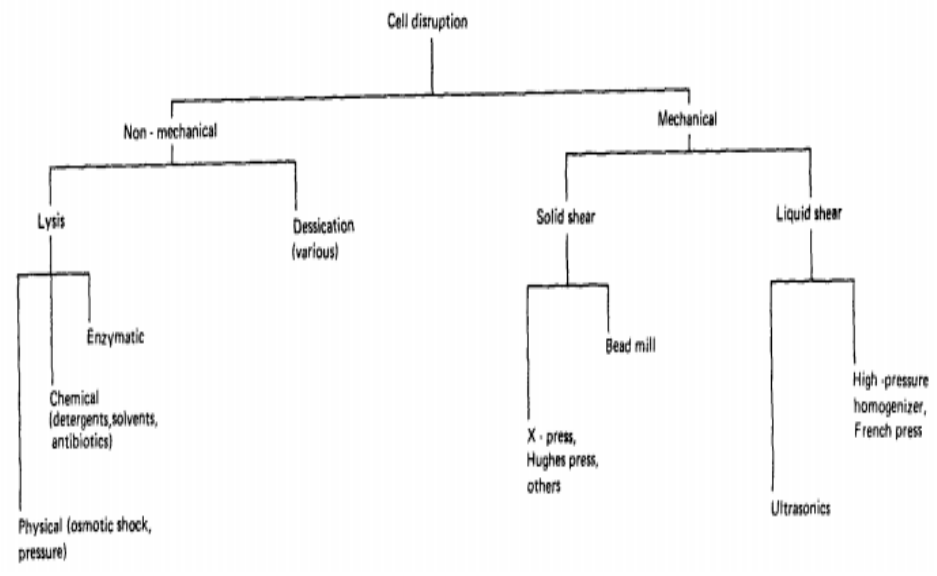
Cell disruption methods

- Microorganisms are more robust than is generally believed. The resistance to disruption of microorganisms has been referred to by Wimpenny.
- He points out that the internal pressure due to osmosis inside an organism such as Micrococcus lysodeikticus or Sarcina lutea is about 20 atmospheres and that the structures responsible for resisting this pressure are about as strong, weight for weight, as reinforced concrete.

- A variety of disruption methods are available to disintegrate these strong cellular walls and membranes and liberate the cell contents.
- A useful classification of the cell disruption methods were shown below.
- Only some, mainly mechanical, cell disruption methods have found industrial application. These will be described in more detail in the latter parts of this review.

Mechanical cell disruption

- Both solid shear (e.g. bead mill) and liquid shear (e.g. high pressure homogenizer) based methods of cell disruption have proven successful on a large scale.
- The solid shear methods may involve either a grinding action as in a ball mill or may involve extrusion of frozen cells, either alone or as a cell-ice (or other abrasive) mixture, through narrow gaps or orifices under high pressure.
- Most of the ceil disruption equipment in current use was originally designed for the homogenization and size reduction of very different commercial products such as milk and paint.



Liquid shear methods

- The high-pressure homogenizer.

- Among the liquid shear disruption devices, the high-pressure Manton-Gaulin APV type homogenizer is probably the most widely used.
- The technical feasibility of this system for cell disruption was demonstrated by Dunnill and Lilly and their coworkers and the use of this equipment has been further reported.
- The high-pressure homogenizer consists of a positive displacement piston pump with one or more plungers.
- The cell suspension is drawn through a check valve into the pump cylinder and, on the pressure stroke, is forced through an adjustable discharge valve with restricted orifice.
- The disruption of bakers' yeast, *Saccharomyces cerevisiae*, in an APV Manton-Gaulin homogenizer was examined by Hetherington and coworkers.
- The 'knife edge' valve seat was found to give higher disruption thanthe 'flat' type unit at the same operating pressure. The disruption followed first-order kinetics and could be described by:

$\log [R_m/(R_m - R)] = k\, NP^a$

where R m is the maximum obtainable protein release after N passes through the valve, k is a dimensional (Pa) disruption rate constant. The exponent, a, on the operating pressure,

Ultrasonication.

- Ultrasonication is another liquid-shear method of disruption which has received some attention in the literature, Ultrasound, sound of frequency higher than 15-20 kHz which is inaudible to the human ear, is known to cause both inactivation and, at higher acoustic power inputs, disruption of microbial cells in suspension.

Solid shear methods

- The bead mill. Cell disruption in bead mills is regarded as one of the most efficient techniques for physical cell disruption.
- Various designs of bead mills have been used formicrobial cell disruption.

- These mills consist of either a vertical or a horizontal cylindrical chamber with a motor-driven central shaft supporting a collection of off centered discs or other agitating element.
- The chamber is filled to the desired level with steel or ballotini glass beads which provide the grinding action.
- The charge of grinding beads is retained in the chamber by a sieve-plate covering the bottom inlet in vertical machines, while in horizontal units the fluid entry is above the level of the beads in the chamber and no retention mechanism is required.
- At the fluid exit port, three different types of bead retention systems have been employed: a sieve-plate, a disc rotating in very close proximity to a plate with a central exit port in its and a vibrating slot.
- The latter two types of bead retention devices are said to reduce fouling problems.
- The horizontal configuration of the mill is known to give a better efficiency of disruption relative to the vertical one.
- This is because the upward fluid flow in vertical machines tends to fluidize the grinding beads to some degree, thereby reducing grinding efficiency.

The freeze-press.

- Freeze-pressing of microbial cell suspensions can be used to disrupt the cells. Examplesof the freeze-pressing equipment include the Hughes press in which a frozen paste of cells is forced through a narrow slit or orifice, either in the presence of an abrasive at temperatures just below zero or without the abrasive at temperatures of about -25°C.
- In the latter case, phase and consequent volume changes of ice contribute to disruption. In addition, solid shear due to crystalline ice is important. According to Wimpenny, cell breakage in the Hughes press yields cell wall membrane preparations that are relatively intact and may be a good method for isolation of membrane-associated enzymes.

- A semi continuous design of X-Press freeze-pressing equipment capable of handling about 10 kg material every hour has been studied.
- For a similar machine, a report suggests that the device can be scaled up almost unlimitedly. The thermodynamics of compression, and rheology in such devices have received theoretical treatment in a number of papers.
- We are not aware of any industrial freeze-pressing equipment commercially available or in use. The reader is referred to other papers for information on this technique.

Foam separation and control

- Foam separation is a chemical process which falls into a category of separation techniques called "Adsorptive bubble separation methods". It is further divided into froth flotation and foam fractionation.
- Foam is a type of colloidal dispersion where gas is dispersed throughout a liquid phase. The liquid phase is also called the continuous phase because it is an uninterrupted, unlike the gas phase.
- Foam is produced during most microbial fermentations. Foaming may occur either due to a medium component, e.g., protein present in the medium, or due to some compound produced by the microorganism. Proteins are present in corn-steep liquor, pharma media, peanut meal, soybean meal, etc.
- These proteins may denature at the air-broth interface and form a protein film that does not rupture readily. Foaming can cause removal of cells from the medium; such cell wills undergo autolysis and release more proteins into the medium. This, in turn, will further stabilize the foam. Five different patterns of foaming are recognized; these are listed below.

1. Foaming remains at a constant level throughout the fermentation. Initial foaming is due to the medium, but later microbial activity contributes to it.

2. Foaming declines steadily in the initial stages, but remains constant thereafter. This type of foaming is due to the medium.

3. The foaming increases after a slight initial fall', in this case, microbial activity is the major cause of foaming.

4. The foaming level increases with fermentation duration; such foaming pattern is solely due to microbial activity.

5. A complex foaming pattern that combines features of two or more of the above patterns.

Foaming may lead to several physical and biological problems. Some examples of physical problems are as follows:

(1) The working volume of the fermenter may decrease due to a circulation of oxygen-depleted gas bubbles in the system.

(2) The bubble size may also decrease, and

(3) The heat and mass transfer rates may also decline.

(4) Foaming may interfere with the functioning of sensing electrodes resulting in invalid process data, and incorrect monitoring and control of pH, temperature, etc.

The biological problems of foaming include

(1) deposition of cells in the upper parts of the fermenter,

(2) (2 problems of sterile operation as the air filter exits of the fermenter become wet, and

(3) (3) increased risk of contamination. In addition,

(4) (4) there may be product loss due to siphoning of the culture broth.

Whenever excessive foaming occurs, the following approaches may be used to resolve the problem:

(1) A defined medium may be used to avoid foam formation. This may be combined with modifications in physical parameters like pH, temperature, aeration and agitation. This approach will be successful in such cases where medium is the main culprit, but will fail whenever microbial activity is the main contributor.

(2) Often the foam may be unavoidable; in such case, antifoam should be used. This is the most standard approach to combat foaming.

(3) A mechanical foam breaker may also be used. Antifoams are surface active agents; they reduce surface tension in the foams and destabilize protein film by the following effects:

(a) hydrophobic bridges between two surfaces,

(b) displacement of the absorbed protein, and

(c) rapid spreading of the surface film.

- Several compounds meet and have been found to be suitable for different fermentation processes; these compounds are as follows: alcohols (stearyl and octyl decanol), esters, fatty acids and their derivatives (especially, triglycerides like cottonseed oil, linseed oil, soybean oil, sunflower oil, etc.), silicones, sulphonates, and miscellaneous compounds like oxaline, Alkaterge C, and polypropylene glycol.
- Many of the antifoams are of low solubility; therefore, they are added with a carrier like lard oil, liquid paraffin and castor oil. There carriers, however, may be metabolized, and they may affect the fermentation process. Further, many antifoams would reduce oxygen transfer by up to 50% when used at effective concentrations.
- Antifoams are generally added when foaming occurs during fermentation. But foam control in fermentation industry is still an empirical art. Therefore, the best method of foam control for a particular process in one factory is not necessarily the best for the same process in other factories.
- Further, the design and operating parameters of the fermenters may affect the properties and the foams produced during the fermentation process.

Precipitation

- Precipitation is the creation of a solid in a solution or inside another solid during a chemical reaction or by diffusion in a solid.

- When the reaction occurs in a liquid solution, the solid formed is called the 'precipitate'. The chemical that causes the solid to form is called the 'precipitant'.
- Without sufficient force of gravity (settling) to bring the solid particles together, the precipitate remains in suspension.
- After sedimentation, especially when using a centrifuge to press it into a compact mass, the precipitate may be referred to as a 'pellet'. The precipitate-free liquid remaining above the solid is called the 'supernate' or 'supernatant'.
- Powders derived from precipitation have also historically been known as 'flowers'.Sometimes the formation of a precipitate indicates the occurrence of a chemical reaction.
- If silver nitrate solution is poured into a solution of sodium chloride, a chemical reaction occurs forming a white precipitate of silver chloride.
- When potassium iodide solution reacts with lead nitrate solution, a yellow precipitate of lead iodide is formed.
- Precipitation may occur if the concentration of a compound exceeds its solubility (such as when mixing solvents or changing their temperature). Precipitation may occur rapidly from a supersaturated solution.
- In solids, precipitation occurs if the concentration of one solid is above the solubility limit in the host solid, due to e.g. rapid quenching or ion implantation, and the temperature is high enough that diffusion can lead to segregation into precipitates. Precipitation in solids is routinely used to synthesize nanoclusters.
- An important stage of the precipitation process is the onset of nucleation. The creation of a hypothetical solid particle includes the formation of an interface, which requires some energy based on the relative surface energy of the solid and the solution.

- If this energy is not available, and no suitable nucleation surface is available, super saturation occurs.

Filtration

- Filtration separates particles by forcing the fluid through a filtering medium on which solids are deposited.
- Filtration can be divided into several categories depending on the filtering medium used, the range of particle sizes removed, the pressure differences, and the principles of the filtration, such as conventional filtration, microfiltration, ultrafiltration, and reverse osmosis.
- In this section we limit our discussion on the conventional filtration which involves large particles. This technique is effective for dilute suspension of large and rigid particles.

Various factors will influence the choice of the most table type of equipment to meet the specified requirements at minimum overall cost, including:

1. The properties of the filtrate, particularly its viscosity and density.
2. The nature of the solid particles, particularly their size and shape, the size distribution and packing characteristics.
3. The solids: liquid ratio.
4. The need for recovery of the solid or liquid fraction or both.
5. The scale of operation.
6. The need for batch or continuous operation.
7. The need for aseptic conditions.
8. The need for pressure or vacuum suction to ensure an adequate flow rate of the liquid.

The following filtration methods to be followed for filtering the content from fermentation broth.

Simple filter

P late and frame filter

Pressure leaf filter

- vertical
- horizontal
- stacked disc filter

Rotary vacuum filter

Simple filter

- A simple filtration consists of a support covered with a porous filter cloth. A filter cake gradually builds up as filtrate passes through the filter cloth.
- As the filter cake increases in thickness the resistance to flow will gradually increase. Thus, if the pressure applied to the surface of the slurry is kept constant the rate of flow will gradually diminish.
- Alternatively, if the flow rate is to be kept constant the pressure will gradually have to be increased. The flow rate may also be reduced by blocking of holes in the filter cloth and closure of voids between particles, if the particles are soft and compressible.
- When particles are compressible it may not be feasible to apply increased pressure.

Flow through a uniform and constant depth porous bed can be represented by the Darcy equation:

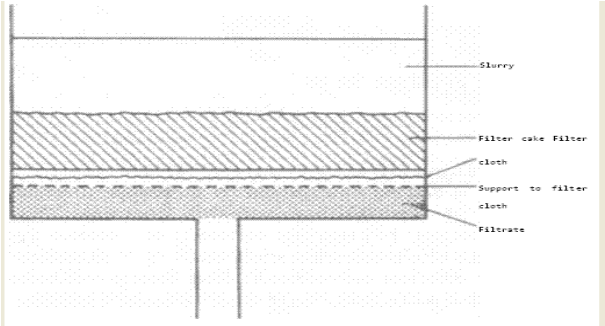
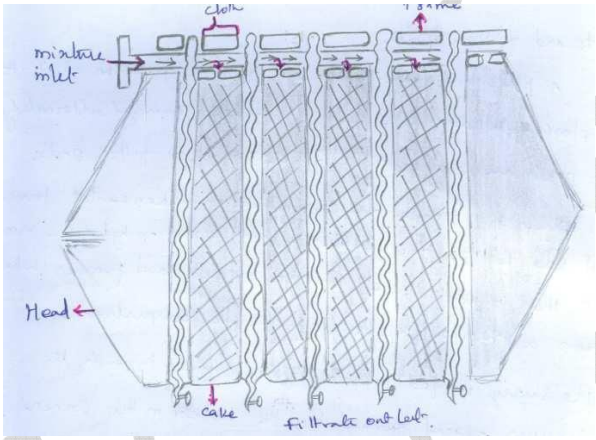


Plate And Frame Filters

- A plate and frame filter is a pressure filter in which lie simplest form consists of plates and frames arranged alternately.
- The plates are covered with filter cloths or filter pads. The plates and frames it assembled on a horizontal framework and held ether by means of a hand screw or hydraulic ram so that there is no leakage between the plates and frames which form a series of liquid-tight compartments.
- The slurry is fed to the filter frame through the continuous channel formed by the holes in the corners of the plates and frames.
- The filtrate passes through the filter doth or pad, runs down grooves in the filter plates and then discharged through outlet taps to a channel. Sometimes, if aseptic conditions are required, the out-lets may lead directly into a pipe.
- The solids are reamed within the frame and filtration is stopped when lie frames are completely filled or when the flow of titrate becomes uneconomically low.
- On an industrial scale the plate and frame filter is one of the cheapest filters per unit of filtering space and requires the least floor space, but it is intermittent in operation (a batch process) and there may be considerable wear of filter cloths as a result of frequent dismantling.

- This type of filter is most suitable for fermentation broths with a low solids content and low resistance to filtration.
- It is widely used as a 'polishing' device in breweries to filter out residual yeast cells following initial clarification by centrifugation or rotary vacuum filtration.
- It may also be used for collecting high value solids that would not justify the use of a continuous filter.
- Because of high labour costs and the time involved in dismantling, cleaning and reassembly, these filters should not be used when removing large quantities of worthless solids from a broth.



Pressure Leaf Filters

- There are a number of intermittent batch filters usually called by their trade names. These filters incorporate a number of leaves, each consisting of a metal framework of grooved plates which is covered with a fine wire mesh, or occasionally a filter cloth and often precoated with a layer of cellulose fibres.

- The process slurry is fed into the filter which is operated under pressure or by suction with a vacuum pump. Because the filters are totally enclosed it is possible to sterilize them with steam.

This type of filter is particularly suitable for 'polishing' large volumes of liquids with low solids content or small batch filtrations of valuable solids.

(i) Vertical metal-leaf filter

- This filter consists of a number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure vessel. The solids from the slurry gradually build up on the surface of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft.
- In some designs the hollow shaft can be slowly rotated during filtration. Solids are normally removed at the end of a cycle by blowing air through the shaft and into the filter leaves.

(ii) Horizontal metal-leaf filter

- In this filter the metal leaves are mounted on a vertical hollow shaft within a pressure vessel. Often, only the upper surfaces of the leaves are porous.
- Filtration is continued until the cake fills the space between the disc-shaped leaves or when the operational pressure has become excessive.
- At the end of a process cycle, the solid cake can be discharged by releasing the pressure and spinning the shaft with a drive motor.

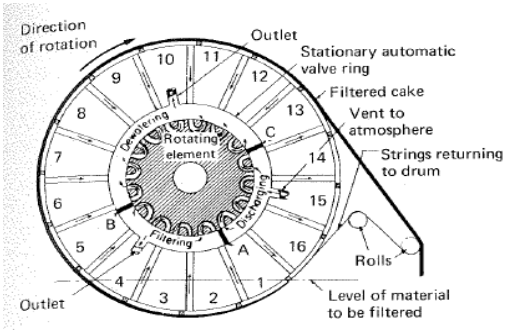
(iii) Stacked-disc filter

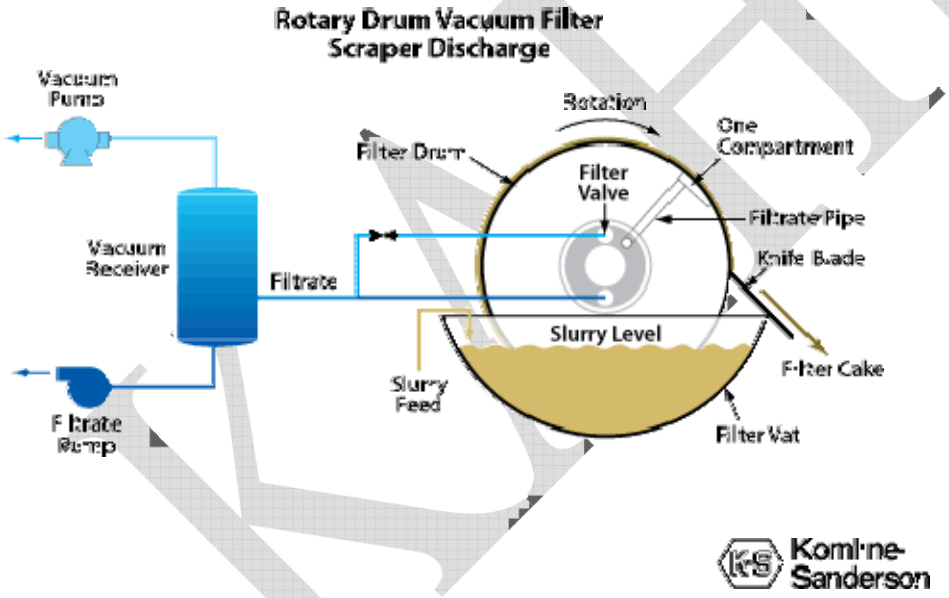
- One kind of filter of this type is the Metafilter.
- This is a very robust device and because there is no filter cloth and the bed is easily replaced, labour costs are low. It consists of a number of precision-made rings which are stacked on a fluted rod. The rings

Rotary vacuum filter

- Large rotary vacuum filter are commonly used by industries which produce large volumes of liquid which need continuous processing.

- The filter consists of a rotating hollow segmented drum covered with a fabric or metal filter whaich is partially immersed in a trough containing the broth to be filtered.
- The slurry is fed on to the outside of the revolving drum and vacuum pressure is applied internally so that the filtrate to a collecting vessels.





The rotary vacuum filter consists of three way of slurry discharge

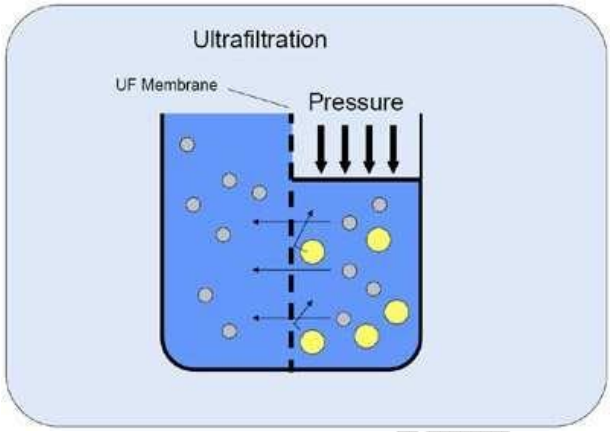
String discharge

Scarper discharge

Scarper discharge with precoating of the drum.

Ultra Filtration

- Ultrafiltration (UF) is a variety of membrane filtration in which forces like pressure or concentration gradients lead to a separation through a semipermeable membrane.
- Suspended solids and solutes of high molecular weight are retained in the so-called retentate, while water and low molecular weight solutes pass through the membrane in the permeate.
- This separation process is used in industry and research for purifying and concentrating macromolecular (103 - 106 Da) solutions, especially protein solutions.



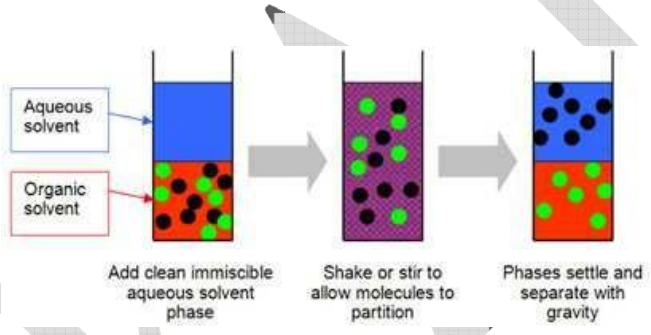
- Ultrafiltration is not fundamentally different from microfiltration. Both of these separate based on size exclusion or particle capture.
- It is fundamentally different from membrane gas separation, which separate based on different amounts of absorption and different rates of diffusion.

Solvent extraction or liquid –liquid extraction

- Liquid–liquid extraction (LLE) consists in transferring one (or more) solute(s) contained in a feed solution to another immiscible liquid (solvent).
- The solvent that is enriched in solute(s) is called extract. The feed solution that is depleted in solute(s) is called raffinate.
- Liquid–liquid extraction also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids,

usually water and an organic solvent. It is an extraction of a substance from one liquid into another liquid phase.

- Liquid–liquid extraction is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separatory funnels to countercurrent distribution equipment. This type of process is commonly performed after a chemical reaction as part of the work-up.
- The term partitioning is commonly used to refer to the underlying chemical and physical processes involved in liquid–liquid extraction, but on another reading may be fully synonymous with it.
- The term solvent extraction can also refer to the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In that case, a soluble compound is separated from an insoluble compound or a complex matrix.



- Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries.
- Liquid–liquid extraction is possible in non-aqueous systems: In a system consisting of a molten metal in contact with molten salts, metals can be extracted from one phase to the other.

- This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam that modifies its electrochemistry greatly.
- For example, it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead, water is reduced to hydrogen. A detergent or fine solid can be used to stabilize an emulsion, or third phase.

Chromatography techniques

- In many fermentation processes, chromatographic techniques are used to isolate and purify relatively low concentrations of metabolic products.
- In this context, chromatography will be concerned with the passage and separation of different solutes as liquid is passed through a column, i.e. *liquid chromatography*. Depending on the mechanism by which the solutes may be differentially held in a column, the techniques can be grouped as follows:

(a) Adsorption chromatography.

(b) Ion-exchange chromatography.

(c) Gel permeation chromatography.

(d) .Affinity chromatography.

(e) Reverse phase chromatography.

(f) High performance liquid chromatography.

Adsorption chromatography

- Adsorption chromatography involves binding of the solute to the solid phase primarily by weak Van de Waals forces. The materials used for this purpose to pack columns include inorganic

adsorbants (active carbon, aluminium oxide, aluminium hydroxide, magnesium oxide, silica gel) and organic macro-porous resins.

- Adsorption and affinity chromatography are mechanistically identical, but are trategically different. In affinity systems selectivity is designed rationally whilst in adsorption selectivity must be determined empirically.
- Di-hydro-streptomycin can be extracted from filtrates using activated charcoal columns. It is then eluted with methanolic hydrochloric acid and purified in further stages. Some other applications for small-scale antibiotic purification are quoted by Weinstein and Wagman (1978). Active carbon may be used to remove pigments to clarify broths. Penicillin-containing solvents may be treated with 0.25 to 0.5% active carbon to remove pigments and other impurities.
- Macro-porous adsorbants have also been tested. The first synthetic organic macro-porous adsorbants, the Amberlite XAD resins, were produced by Rohm and Haas in 1965. These resins have surface polarities which vary from non-polar to highly polar and do not possess any ionic functional groups. Voser (1982) considers their most interesting application to be in the isolation of hydrophilic fermentation products. He stated that these resins would be used at Ciba-Geigy in recovery of cephalosporin C (acidic amino acid), cefotiam (basic amino acid), desferrioxamine B (basic hydroxamic acid) and paramethasone (neutral steroid).

Ion exchange

- Ion exchange can be defined as the reversible exchange of ions between a liquid phase and a solid phase (ion-exchange resin) which is not accompanied by any radical change in the solid structure. Cationic ion-exchange resins normally contain a suiphonic acid, carboxylic acid or phosphonic acid active group: Carboxy- methyl cellulose is a common cation exchange resin.
- Positively charged solutes (e.g. certain proteins) will bind to the resin, the strength of attachment de pending on the net charge of the solute at the pH of the column feed. After deposition solutes

are sequentially washed off by the passage of buffers of increasing ionic strength or pH. Anionic ion-exchange resins normally contain a secondary amine, quaternary amine or quaternary ammonium active group.

- A common anion exchange resin, DEAE (diethylaminoethyl) cellulose is used in a similar manner to that described above for the separation of negatively charged solutes. Other functional groups may also be attached to the resin skeleton to provide more selective behaviour similar to that of affinity chromatography.
- The appropriate resin for a particular purpose will depend on various factors such as bead size, pore size, diffusion rate, resin capacity, range of reactive groups and the life of the resin before replacement is necessary. Weak-acid cation ion-exchange resins can be used in the isolation and purification of streptomycin, neomycin and similar antibiotics.

Gel permeation

- This technique is also known as gel exclusion and gel filtration. Gel permeation separates molecules on the basis of their size.
- The smaller molecules diffuse into the gel more rapidly than the larger ones, and penetrate the pores of the gel to a greater degree.
- This means that once elution is started, the larger molecules which are still in the voids in the gel will be eluted first.
- A wide range of gels are available, including cross-linked dextrans (Sephadex and Sephacryl) and cross-linked agarose (Sepharose) with various pore sizes depending on the fractionation range required.
- One early industrial application, although on a relatively small scale, was the purification of vaccines. Tetanus and diphtheria broths for batches of up to 100,000 human doses are passed through a 13 dm^3 column of G 100 followed by a 13 dm^3 column of G 200.

- This technique yields a fairly pure fraction which is then concentrated ten-fold by pressure dialysis to remove the eluant buffer (Na_2HP0_4).

Affinity chromatography

- Affinity chromatography is a separation technique with many applications since it is possible to use it for separation and purification of most biological molecules on the basis of their function or chemical structure.
- This technique depends on the highly specific interactions between pairs of biological materials such as enzyme-substrate, enzyme-inhibitor, antigen-antibody, etc.
- The molecule to be purified is specifically adsorbed from, for example, a cell lysate applied to the affinity column by a binding substance (ligand) which is immobilized on an insoluble support (matrix). Eluent is then passed 'through the column to release the highly purified and concentrated molecule.
- The ligand is at tached to the matrix by physical absorption or chemically by a covalent bond.

  The pore size and ligand location must be carefully matched to the size of the product for effective separation. The latter method is preferred whenever possible.
- Coupling procedures have been developed using cyanogen bromide, bisoxiranes, disaziridines and periodates, for matrixes of gels and beads. Four polymers which are often used for matrix materials are agarose, cellulose, dextrose and polyacrylamide. Agarose activated with cyanogen bromide is one of the most commonlyused supports for the coupling of amino ligands.
- Silica based solid phases have been shown to be an effective alternative to gel supports in affinity chromatography.

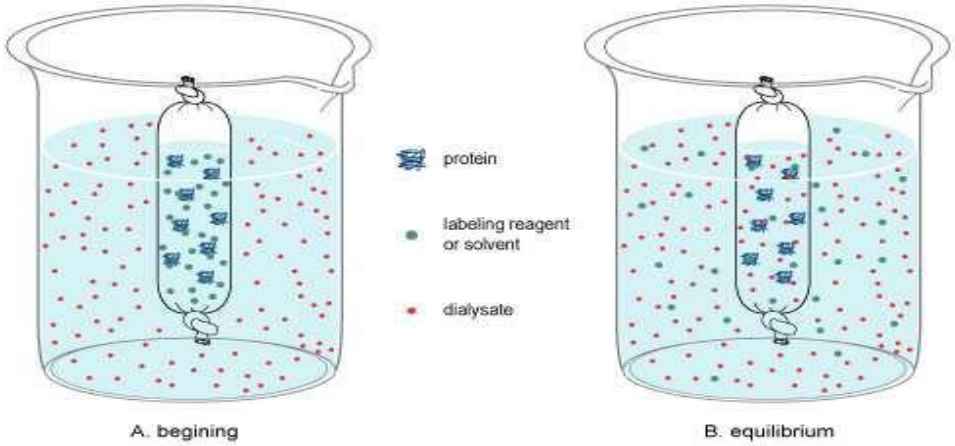
- Purification may be several thousand-fold with good recovery of active material. The method can however be quite costly and time consuming, and alternative affinity methods such as affinity cross-flow filtration, affinity precipitation and affinity partitioning may offer some advantages.
- Affinity chromatography was used initially in protein isolation and purification, particularly enzymes. Since then many other large-scale applications have beendeveloped for enzyme inhibitors, antibodies, interferon and recombinant proteins and on a smaller scale for nucleic acids, cell organelles and whole cells.
- In the scale-up of affinity chromatographic processes bed height limits the superficial velocity of the liquid, thus scale-up requires. An increase in bed diameter or adsorption capacity.

Reverse phase chromatography (RPC)

- This chromatographic method utilizes a solid phase (e.g. silica) which is modified so as to replace hydrophilic groups with hydrophobic alkyl chains. This allows the separation of proteins according to their hydrophobicity.
- More-hydrophobic proteins bind most strongly to the stationary phase and are therefore eluted later than less-hydrophobic proteins.
- The alkyl groupings are normally eight or eighteen carbons in length (C, and CIS)' RPC can also be combined with affinity techniques in the separation of, for example, proteins and peptides.
- Chromatographic techniques are also used in the final stages of purification of a number of products. The scale-up of chromatographic processes can prove difficult, and there is much current interest in the use of mathematical models and computer programmes to translate data obtained from small-scale processes into operating conditions for larger scale applications.

Dialysis

- In biochemistry, dialysis is the process of separating molecules in solution by the difference in their rates of diffusion through a semi permeable membrane, such as dialysis tubing.



- The most common application of dialysis is for the removal of unwanted small molecules such as salts, reducing agents, or dyes from larger macromolecules such as proteins, DNA, or polysaccharides. Dialysis is also commonly used for buffer exchange and drug binding.

Equipment

Separating molecules in a solution by dialysis is a straightforward process. Other than the sample and dialysate buffer, all that is typically needed is:

- Dialysis membrane in an appropriate format (e.g., tubing, cassette, etc.) and molecular weight cut-off (MWCO)
- A container to hold the dialysate buffer
- The ability to stir the solutions and control the temperature (optional)

General Protocol

- A typical dialysis procedure for protein samples is as follows:
- Prepare the membrane according to instructions
- Load the sample into dialysis tubing, cassette or device
- Place sample into an external chamber of dialysis buffer (with gentle stirring of the buffer)

- Dialyze for 2 hours (at room temperature or 4 ℃)
- Change the dialysis buffer and dialyze for another 2 hours
- Change the dialysis buffer and dialyze for 2 hours or overnight

Centrifugation

- Microorganism and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method.
- Although a centrifuge may be expensive when compared with a filter it may be essential when filtration is slow and difficult The cells or other suspended matter must be obtained free of filter aids Continuous separation to a high standard of hygiene is required
- Non- continuous centrifuges are of extremely limited capacity and therefore not suitable for large scale separation
- The centrifuges used in harvesting fermentation broths are all operated on a continuous or semi continuous basis.
- Some centrifuge can be used for separating two immiscible liquids yielding a heavy phase and light phase liquid as well as a solids fraction.
- According to the Stoke's Law, the rate of sedimentation of spherical particles suspended in a fluid of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles, thus the rate of sedimentation of a particle under gravitational force is

$$V_g = \frac{d^2 g\,(\rho_P - \rho_L)}{18\mu}$$

where V_g = rate of sedimentation (m s^{-1})
d = particle diameter (m)
g = gravitational constant (m s^{-2})
ρ_P = particle density (kg m^{-3})
ρ_L = liquid density (kg m^{-3})
μ = viscosity (kg m^{-1} s^{-1})

This equation can then be modified for sedimentation in a centrifuge:

$$V_c = \frac{d\omega^2 r\,(\rho_P - \rho_L)}{18\mu}$$

- When filtration is not a satisfactory separation method, a centrifuge can be used to remove microorganisms and other similar size particles, from a broth.
- It's more expensive, comparing with a filter, but may be essential when filtration is slow and difficult.
- The cells or other suspended matters must be obtained free of filter aids.
- Continuous separation to a higher standard of hygiene is required.

Cetrifugation efficiency is favoured by:

large particle diameter of cells

large density difference between cell and liquid

the liquid should have a low viscosity

In practice, particles of biological material are often small and of low density, which fermentation broths are often viscous, and high density.

It requires :

high angular velocity

large radius(of centrifuge)

large volume

thin sedimentation layer

- Centrifugation is a process that involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, used in industry and in laboratory settings. This process is used to separate two immiscible liquids.
- Centrifugation is used to separate materials of different density when a force greater than gravity is desired

The type of industrial centrifugation unit:

Separate the solid and liquid matter of the mixture using centrifugal force. When we give the centrifugal force, the solid material will be settled down at the bottom of the container and liquid material stay on the above solid phase (pellet).

Basket centrifuge

They used for food.

Speed limit up to 4000 rpm

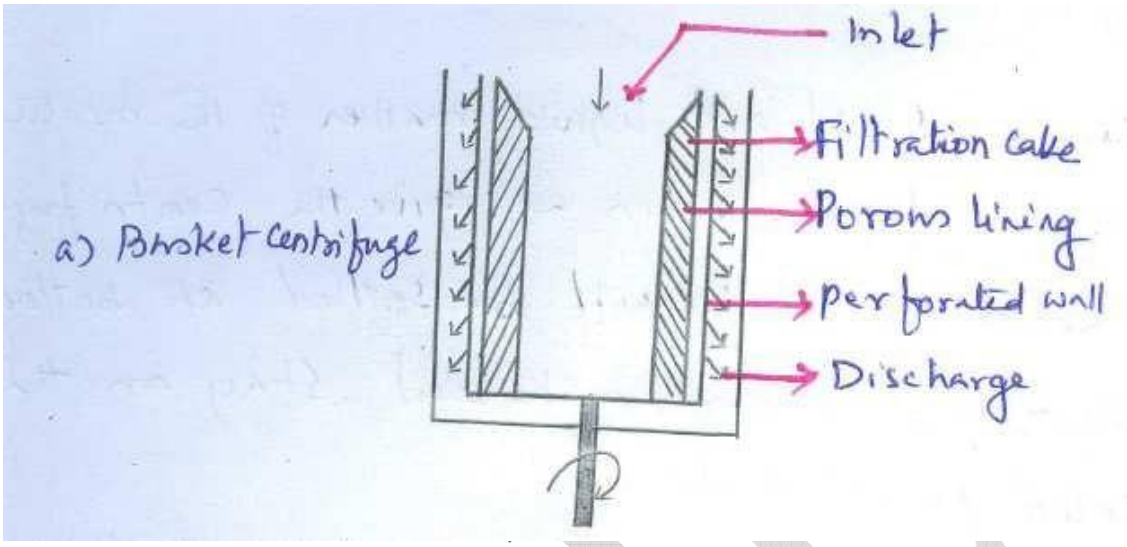
Simple centrifugation process using this can remove the course particles from the fermented media.

Mainly used for separation of mould mycelia and crystalline compounds.

The centrifuge is most commonly used with a perforated bowl lined with a filter bag of nylon, cotton etc.,

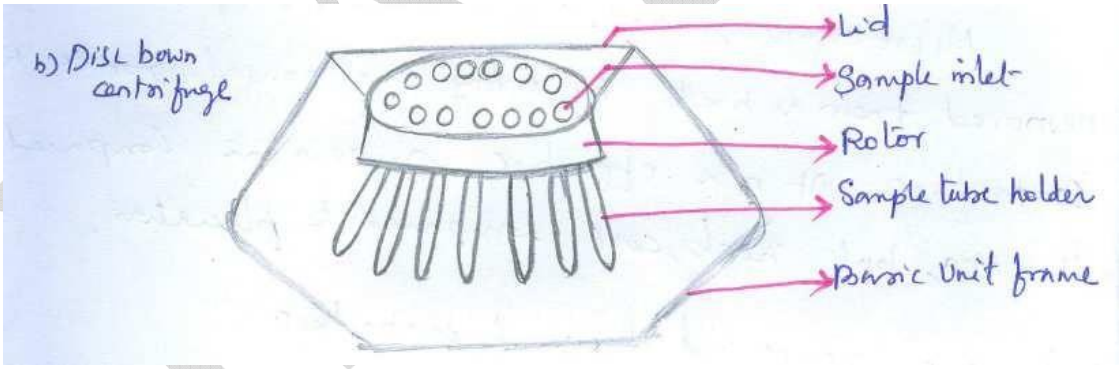
A continuous feed is used and when the basket is filled with the filter cake, is is possible to wash the cake before removing it.

The basket centrifuge may be considered to be a centrifugal filter.



Disc bowl centrifuge

Disc-stack bowl centrifuge. This type is common in bioprocess. The developed forces is 5000-15000 G with minimal density difference between solid and liquid is 0.01-0.03 kg/m3. The minimum particle diameter is 5 µ m

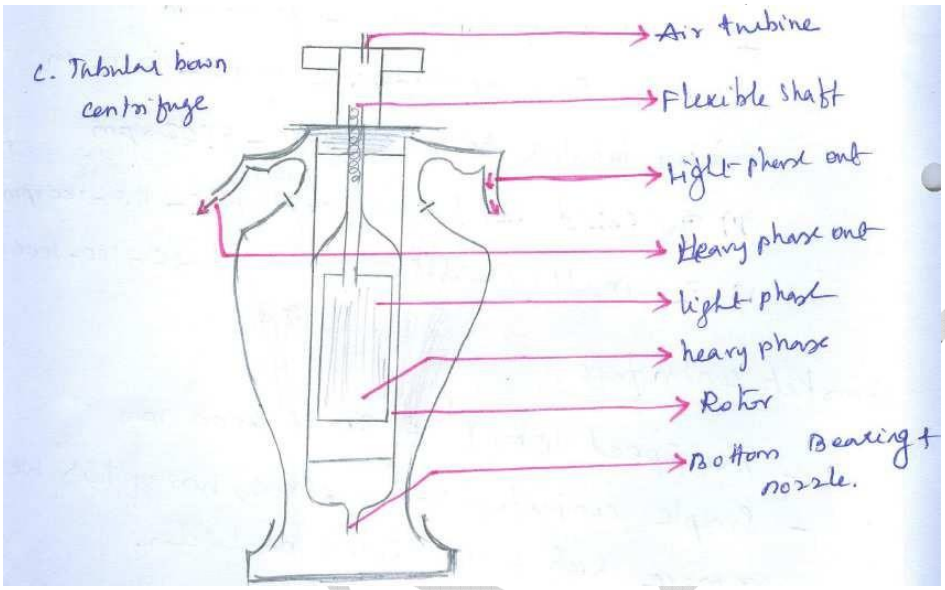


The tubular bowl centrifuge

Hazards to the enzyme are aeration and the consequent foaming of the clarified solution, which aerosol formation may be a hazard to the user. This occurs because of turbulence in the bowl.

Very high angular velocity ⓪turbulence ⓪foaming & aerosol

Tubular bowl centrifuge (Narrow tubular bowl centrifuge or ultracentrifuge, decanter centrifuge, etc). Simple and widely applied in food and pharmaceutical industry. Operates at 13000-16000 G, 105-106 G for ultracentrifuge



- This is a centrifuge to consider using for particle size ranges of 0.1 to 200µm and up to 10% solids in the ingoing slurry.
- The main components of the centrifuge is a cylindrical bowl (rotor) which may be a variable design depending on application suspended by a flexible shaft, driven by an overhead motor or air turbineThe centrifuge may be altered Light phase / heavy phase and liquid phase separation.

The solid bowl scroll centrifuge

- They used for continuously handling coarse material (such as sewage sludge. They're few hazards to enzymes.)

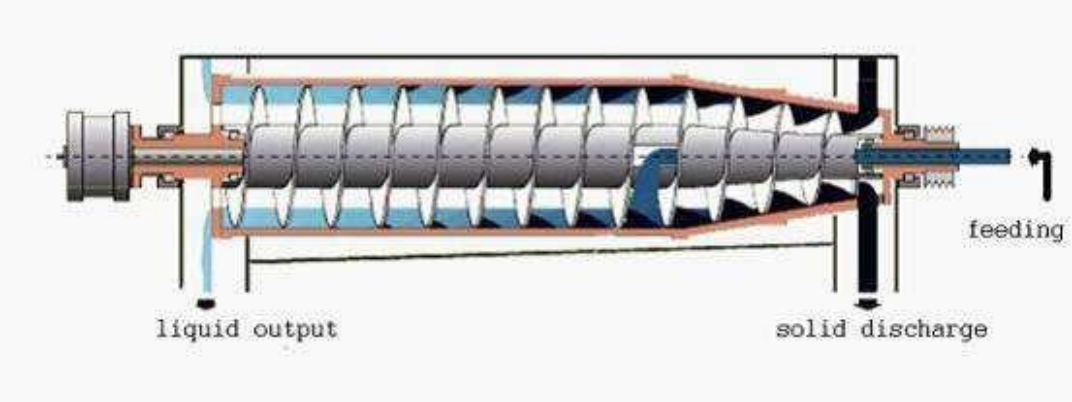
The multi chamber centrifuge

- They have large radius, low angular velocity, thus sedimentation occurs with high efficiency over large surface area.
- They're widely used for Baker's yeast. Some heating(because the bowl is located above the gearbox) represents a danger to the enzyme.

Wastewater Treatment Decanter Centrifuge

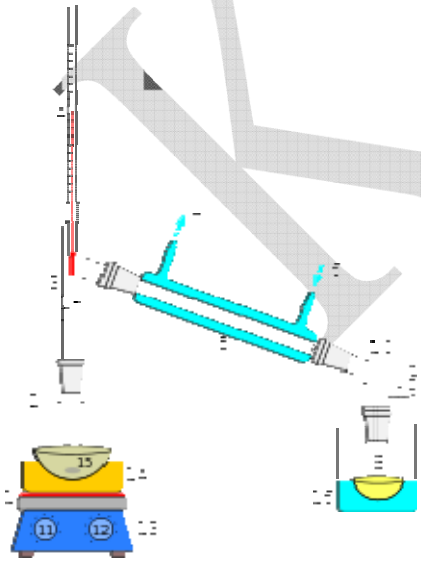
- Whole waste water treatment includes sludge feeding system, flocculent adding system, dewatering system, filter cake delivery system and PLC control system.
- The main equipment of sludge dewatering complete system is decanter centrifuge.
- There are basically two application types in the wastewater treatment industry for the decanter centrifuge: sludge thickening and sludge dewatering duties.
- Also .it can be used for Sludge classification and Sludge clarification. With the decanter centrifuge, it is possible to separate the particles from suspending liquid with equivalent diameter bigger than 0.003mm,solid-liquid weight ratio less than 10% or volume ratio less than 70%,difference in specific gravity bigger than 0.05g/cm3

- Sludge dewatering complete system could be used in the following fields:
- Municipal sludge treatment, paper mill sludge, starch factory waste water treatment, steel mill sludge, printing and dyeing mill sludge, water works sludge, pharmaceutical waste sludge, PVC sludge, sewage de-sulfurization sludge, grain spillage, power plant ash sludge, dairy sludge, beer sludge, drilling fluid ,electroplating liquid, fermented liquid manure, oil refining sludge, soap sludge, leather sludge and etc.



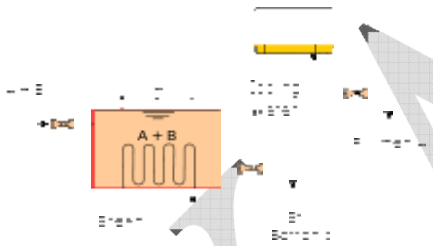
Distillation

- Distillation is a process of separating the component substances from a liquid mixture by selective evaporation and condensation.
- Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of mixture's components.
- In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction.



Batch distillation

- Heating an ideal mixture of two volatile substances A and B (with A having the higher volatility, or lower boiling point) in a batch distillation setup (such as in an apparatus depicted in the opening figure) until the mixture is boiling results in a vapor above the liquid which contains a mixture of A and B.
- The ratio between A and B in the vapor will be different from the ratio in the liquid: the ratio in the liquid will be determined by how the original mixture was prepared, while the ratio in the vapor will be enriched in the more volatile compound, A. The vapor goes through the condenser and is removed from the system.
- This in turn means that the ratio of compounds in the remaining liquid is now different from the initial ratio (i.e., more enriched in B than the starting liquid).



- The result is that the ratio in the liquid mixture is changing, becoming richer in component B.
- This causes the boiling point of the mixture to rise, which in turn results in a rise in the temperature in the vapor, which results in a changing ratio of A : B in the gas phase (as distillation continues, there is an increasing proportion of B in the gas phase). This results in a slowly changing ratio A : B in the distillate.
- If the difference in vapor pressure between the two components A and B is large (generally expressed as the difference in boiling points), the mixture in the beginning of the

distillation is highly enriched in component A, and when component A has distilled off, the boiling liquid is enriched in component B.

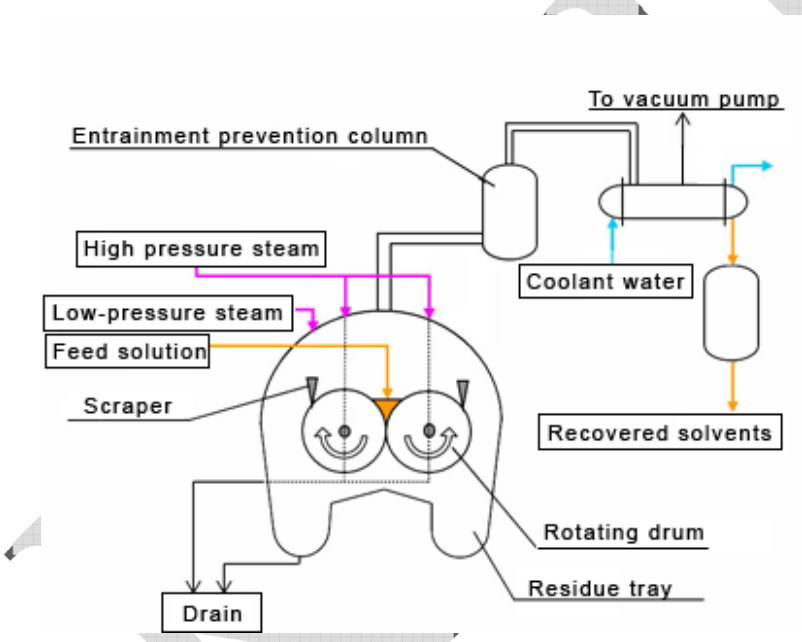
Continuous distillation

- Continuous distillation is an ongoing distillation in which a liquid mixture is continuously (without interruption) fed into the process and separated fractions are removed continuously as output streams occur over time during the operation.
- Continuous distillation produces a minimum of two output fractions, including at least one volatile distillate fraction, which has boiled and been separately captured as a vapor, and then condensed to a liquid. There is always a bottoms (or residue) fraction, which is the least volatile residue that has not been separately captured as a condensed vapor.
- Continuous distillation differs from batch distillation in the respect that concentrations should not change over time. Continuous distillation can be run at a steady state for an arbitrary amount of time.
- For any source material of specific composition, the main variables that affect the purity of products in continuous distillation are the reflux ratio and the number of theoretical equilibrium stages, in practice determined by the number of trays or the height of packing. Reflux is a flow from the condenser back to the column, which generates a recycle that allows a better separation with a given number of trays.
- Equilibrium stages are ideal steps where compositions achieve vapor–liquid equilibrium, repeating the separation process and allowing better separation given a reflux ratio. A column with a high reflux ratio may have fewer stages, but it refluxes a large amount of liquid, giving a wide column with a large holdup. Conversely, a column with a low reflux ratio must have a large number of stages, thus requiring a taller column.

Drying

Drum drying

- Drum drying is a method used for drying out liquids from raw materials with drying drum. In the drum-drying process, pureed raw ingredients are dried at relatively low temperatures over rotating, high-capacity drums that produce sheets of drum-dried product.
- This product is milled to a finished flake or powder form. Modern drum drying techniques results in dried ingredients which reconstitute immediately and retain much of their original flavor, color and nutritional value.



- Some advantages of drum drying include the ability of drum dryers to dry viscous foods which cannot be easily dried with other methods. Drum dryers can be clean and hygienic and easy to operate and maintain.
- Other products where drum drying can be used are for example starches, breakfast cereals, baby food, instant mashed potatoes to make them cold water soluble.

Crystallization

- Crystallization is the (natural or artificial) process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas.
- Crystallization is also a chemical solid–liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs. In chemical engineering crystallization occurs in a crystallizer.
- Crystallization is therefore an aspect of precipitation, obtained through a variation of the solubility conditions of the solute in the solvent, as compared to precipitation due to chemical reaction
- The crystallization process consists of two major events, nucleation and crystal growth.

  Nucleation is the step where the solute molecules dispersed in the solvent start to gather into clusters, on the nanometer scale (elevating solute concentration in a small region), that become stable under the current operating conditions. These stable clusters constitute the nuclei.
- However, when the clusters are not stable, they dissolve. Therefore, the clusters need to reach a critical size in order to become stable nuclei. Such critical size is dictated by the operating conditions (temperature, supersaturation, etc.).
- It is at the stage of nucleation that the atoms arrange in a defined and periodic manner that defines the crystal structure — note that "crystal structure" is a special term that refers to the relative arrangement of the atoms, not the macroscopic properties of the crystal (size and shape), although those are a result of the internal crystal structure.
- The crystallization process performs to know the exact structure of the contents which isolated from the fermented broth as end product.

Fermented product in market

- Fermented foods produced or preserved by the action of microorganisms and refers to the fermentation of sugar to alcohol using yeast, but other fermentation processes involve the use of

bacteria such as lactobacillus, including the making of foods such as yogurt and sauerkraut. The science of fermentation is known as zymology.

- Many pickled or soured foods are fermented as part of the pickling or souring process, but many are simply processed with brine, vinegar, or another acid such as lemon juice.
- Live cultured pickles, sauerkraut, vegetables and kimchi
- Cheese made from raw milk
- Unpasteurized miso
- Tempeh, made from fermented soybeans
- Fermented drinks and tea such as kombucha
- Yogurt and kefir made with live cultures.

Questions	opt1	opt2	opt3	opt4	Answer
UNIT I					
Lag phase is also known as	period of initial	transitional period	generation time	doubling time	period of initial adjustment
In Latin the term ‘fervere” means to _____.	Oxidize	Boil	Polymerize	Reduce	Boil
What is meant by submerged fermentation?	Growing of microbes on moist solid particles or surface	Growing of microbes on nutrient broth	Growing of microbes on solid and liquid	Growing microbes on water	Growing of microbes on nutrient broth
Which of the statement is true for continuous reactor at steady state?	The rates of biomass, substrate and product concentrations are zero	Biomass, substrate and product concentrations do not change with time	Biomass, substrate and product concentrations change with time	changes occur in production level	Biomass, substrate and product concentrations do not change with time
The process of converting a compound in to a structurally related, financially more valuable compound using microbial cells is called as _____.	oxidation	remediation	biotransformation	reduction	biotransformation
_____ is a closed culture system which contains an initial limited amount of	continuous culture	batch culture	external feedback culture	fed-batch culture	batch culture
Time required to double the quantity of biomass is called?	optimum time	doubling time	optimum and doubling time	product time	doubling time
_____ fermentation do not require aeration for culturing microbes for product production.	aerobic fermentation	Batch fermentation	Anaerobic fermentation	fed-batch fermentation	Anaerobic fermentation
A continuous bioreactor in which only the flow rate is used to control the rate of cell or product productivity is called	turbidostat	chemostat	level stat	pH	chemostat
For organisms growing in a chemostat, the specific growth rate	cannot be determined	can be determined from the dilution rate	equals to the maximum specific growth rate of the	growth rate	can be determined from the dilution rate
In aerobic yeast fermentation for production of citric acid from alkanes using a fed-batch culture, why alkanes are slowly fed to the yeast?	Citric acid is toxic to the cells	Alkanes cause foaming	Fast addition of alkanes will inhibit the cells and reduce oxygen transfer rates	Fast addition of alkanes will cause the cells to grow too quickly	Fast addition of alkanes will inhibit the cells and reduce oxygen transfer rates
Which of the following is used to calculate mass of substrate in the reactor?	Flow rate x substrate concentration in the reactor	Volume of reactor x substrate concentration in reactor	Flow rate x mass of reactor	Volume of reactor x Flow rate	Volume of reactor x substrate concentration in reactor
Low dissolved oxygen concentrations leads to	low biomass yields	high biomass yields	no effect on biomass yields	substrate consumption decrease	low biomass yields
Yield coefficient represents	total biomass or product produced	conversion efficiency of a substrate into product	conversion rate of a substrate into biomass or	production time of biomass or product	conversion efficiency of a substrate into product
A continuous bioreactor in which only the flow rate is used to control the rate of cell or product productivity is called	turbidostat	chemostat	level stat	pH	chemostat
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Low dissolved oxygen concentrations leads to	low biomass yields	high biomass yields	no effect on biomass yields	substrate consumption increase	low biomass yields
The main reason for production of antibiotics in fed batch reactors is	precursor	higher yields when cells enter the stationary phase	elucitors	promotors	higher yields when cells enter the stationary phase
A fed-batch reactor initially contains 2 litre of medium. If it was fed at 1 litre per hour, then after 10 hours, the volume of the reactor will be	1 litre	2 litre	3 litre	13 litre	2 litre
The function of the disengagement zone in an airlift fermenter is to	prevent co2 rich bubbles from entering the downcomer	reduce the velocity of the bubbles	reduce liquid loss as aerosols	prevent co2 rich bubbles from entering the downcomer, reduce the velocity of the bubbles, reduce liquid loss as	prevent co2 rich bubbles from entering the downcomer, reduce the velocity of the bubbles, reduce
Stationary phase is described as	no further increase in the cell population after a maximum value	deceleration of growth and division rate after the growth rate reaches a	acceleration of growth and division rate after the growth rate reaches a	deceleration of growth and division rate after the growth rate reaches a minimum	no further increase in the cell population after a maximum value
In the accelerated phase, cell starts to	increase and the division rate increases to reach a maximum	decrease and the division rate increases to reach a maximum	increase and the division rate decreases to reach a maximum	increase and the division rate increases to reach a minimum	increase and the division rate increases to reach a maximum

The function of a mechanical seal is to	prevent contaminants entering the reactor	prevent cells from leaving the reactor	both (a) and (b)	prevent air to enter	both (a) and (b)
The phenomenon in which substrates are used in a sequential manner is known	trans-substrate genesis	dualism	diauxie	multiplicity	diauxie
Diauxie is	growth factors	microbiological die off	the simultaneous uptake of nutrients	the stagewise uptake of nutrients	the stagewise uptake of nutrients
An open system in which the growth rate is maintained by adding a nutrient (present in limiting quantities) at the same rate as that medium containing micro-organisms is removed is called	manostat	chemostat	turbidostat	culturostat	chemostat
In the death phase	nutrients available for the cells are depleted and begin to die	the number of viable cells will increase	nutrients available for cells are replenished and start to multiply	number of dead cells increase	nutrients available for the cells are depleted and begin to die
The maximum specific growth rate of an organism depends on	medium composition	temperature	pH	Medium composition, temp, pH	Medium composition, temp, pH
Bubble column reactor has	large height to diameter	small height to diameter	large diameter to height	small diameter to height ratio	large height to diameter ratio
Why a T-flask used in small-scale cell culture is incubated in a horizontal position?	to save space	to increase the surface area of the liquid-air	both (a) and (b)	to increase the rate of oxygen transfer into the liquid	both (a) and (b)
An open system in which the growth rate is maintained by the removal and addition of media at such a rate as to maintain a constant cell density is called a	manostat	chemostat	turbidostat	culturostat	turbidostat
Wash out in steady state fermentation occurs when	dilution rate is less than maximum specific growth rate	dilution rate is higher than the maximum specific growth rate	cell concentration reaches the maximum	specific growth rate is maximum	dilution rate is higher than the maximum specific growth rate
Fermenter should be filled with medium upto	65-70%	70-75%	75-80%	80-85%	75-80%
Bacterial growth curve is obtained by plotting	number of cells versus time	number fo spores versus time	log of number of cells versus time	log of number of cells survived versus time	log of number of cells versus time
When intracellular enzymes of whole cells are to be used in a bio-conversion process, it is often necessary to_____the cells.	permeabilize	lyophilize	heat-kill	denature	permeabilize
The height to diameter ratio (H/D) for the column fermenters is	< 3	> 3	< 1.5	> 1.5	> 3
The high oxygen transfer efficiencies of airlift bioreactors is due to	the large aspect ratio of the reactor, which leads to a high gas hold-up	the large aspect ratio of the reactor, which leads to a high oxygen solubility at the base of	the draft tube, which reduces bubble coalescence in the reactor	all of the above	all of the above
Population doubling time, td can be expressed as (where μ is the specific	log2/μ	ln2/μ	μ/ln2	μ/log2	ln2/μ
In an airlift bioreactor, the spent gases released from the liquid are called	downcomer	disengagement zone	air riser	flotsam	disengagement zone
The specific growth rate (μ) is defined as	the concentration of biomass in the reactor	rate of increase of total biomass in a reactor	the rate of individual cells division or increase in their biomass	the rate of cell death	the rate of individual cells division or increase in their biomass
The number of baffles in a standard stirred tank bioreactor is	8	6	4	2	4
In the stationary phase	growth is proportional to death	growth rate is equal to death rate	growth rate is lesser than death rate	no co-relation exist between death rate and growth rate	growth rate is equal to death rate
Biomass concentrations during fermentation is	diluting the samples to optical density less than 0.3	monitored by controlling the changes in biomass concentrations	considering that optical density is proportional to the surface area of the biomass in the sample	all of the above	all of the above
Chemostats work on the principle of	maintaining constant volume of culture	maintaining continuous flow of nutrients	maintaining uniform nutrients concentration	operating at higher pressure	maintaining uniform nutrients concentration
The static level of nutrients (chemical) in a bioreactor is known as	manostat	turbidostat	chemostat	culturostat	chemostat
Relation between the turbidity of the culture and the dilution rate in the fermentation vessel are called _____	manostat	turbidostat	chemostat	culturostat	turbidostat
A culture in a closed vessel to which no additional medium is added and from which no waste products are removed is called a _____ culture.	continuous	Batch	fed-batch	semi continuous	batch
Lag phase occurs mainly due to	acclimatization with new environment and	higher growth temperature	substrate disliking	variation in substrate concentration	acclimatization with new environment and substrate

Turbidostat is recommended when continuous fermentation needs to be carried out at	high dilution rates near the washout point	low dilution rates near the washout point	moderate dilution rates near the washout point	any intensity of dilution rates near the washout point	high dilution rates near the washout point
Stirred tank fermenter (STF) can be employed for	aerobic fermentation of a wide range of cells including microbial, animal and plant cells	anaerobic fermentation of a wide range of cells including microbial, animal and plant cells	both (a) and (b)	anaerobic fermentation of plant cells only	both (a) and (b)
The specific growth rate is affected by	sabstrate concentration	product concentration	oxygen supply	all of these	all of these
Which of the following is incorrect?	the mechanical agitation and aeration are effective for suspension	the mechanical agitation and aeration are effective for mixing the medium	STF can't be used for high viscosity medium	the mechanical agitation and aeration are effective for oxygenation. c	STF can't be used for high viscosity medium
The contents in bubble column and air lift reactor are mixed through	compressed air	a mechanical internal moving part	external pumping	sparger	compressed air
During the lag phase	cells just start growing	cells synthesize new proteins and membrane	cells produce antibiotics to kill competing organisms	cells simply grow slowly	cells synthesize new proteins and membrane components
Concentration of the growth limiting substrate means	trace elements concentration	the concentration of the substrate that controls the growth rate of the	sugar concentration in the fermentation medium	dissolved oxygen concentration	the concentration of the substrate that controls the growth rate of the cells
Tower fermentors are used for	continuous penicillin production	continuous beer production	production of enzymes	batch production of beer	continuous beer production
In wastewater treatment, which bioreactor is generally used?	plug flow reactor	trickle bed reactor	tower fermentor	CSTR	trickle bed reactor
The contents in stirred tank rector are mixed through	compressed air	a mechanical agitator	external pumping	sparger	a mechanical agitator
The relatively large volume (10 to 40% of total fermenter volume) of the seed culture is employed to	increase the overall fermentation time	increase the overall yield of biomass	decrease the overall fermentation time	decrease the overall yield of product	decrease the overall fermentation time
Unit II					
In an airlift bioreactor, the air sparging region is called	downcomer	disengagement zone	air riser	sparger	air riser
The region of an airlift bioreactor in which the liquid travels in a downward direction is called the	downcomer	disengagement zone	air riser	flotsam	downcomer
The maximum specific growth rate (μm) of an organism in batch culture is equal to slope of a plot of	in [biomass] against time for exponential phase	in [biomass] against time	biomass against time	biomass against time for stationary phase data only	in [biomass] against time for exponential phase data only
At the end of the lag phase, when growth begins, the division rate increases gradually and reaches a maximum value in the exponential growth period. This transitional period is commonly called as _____	lag phase	accelerated growth phase	exponential growth phase	stationary phase	accelerated growth phase
Chemical transformation of complex organic substances into simpler compounds by the action of enzymes is known as	Fermentation	Oxidation	Dehydration	reduction	Fermentation
_____ involves the freezing of a culture followed by its drying under	drying	crystallization	lyophilization	refrigeration	lyophilization
_____ involves obtaining either pure or mixed cultures followed by their assessment to determine which carry out the desired reaction or produce the	enumeration	contamination	preservation	isolation	isolation
Compound synthesis in the cell and released to the media such compound are called?	Intra cellular compounds	extra cellular compounds	intra and extra cellular compounds	secondary metaboltes	extra cellular compounds
Compound synthesis in the cell and released by cell disruption method only such compounds are called _____	Intra cellular compounds	extra cellular compounds	both types of compounds	biomass against time for stationary phase data only	Intra cellular compounds
Process that, individual cells divide as two daughter cells arise from a single cell are called_____	Binary fission	Biomass	Growth	binary fussion	Binary fission
Contains a single bacterial species and the growth of only one microorganism in a culture is known as _____	enrichment culture	pure culture	mixture of culture	quality culture	pure culture
Culture of microbes on free flowing liquid substrates for the production of metabolites are _____	solid state fermentation	submerged fermentation	immobilized culture	batch fermentation	submerged fermentation
Process that effectively kills or eliminate transmissible agents such as fungi, bacteria and virus from a surface of vessels are _____	sterilization	contamination	decontamination	production	sterilization
The microorganism living under low temperature (<15^0 C) is called ……	Thermophiles	Mesophiles	psychrophiles	hygrophiles	psychrophiles
The microorganism living under low temperature (25 - 40^0 C) is known as …	Thermophiles	Mesophiles	psychrophiles	hygrophiles	Mesophiles
Who published the book entitle "*Studies on Fermentation" ?*	Guy-Lussac	Eduard Buechner	Louis Pasteur	George Stahl	Louis Pasteur

What is the expanded form of ATCC?	American tissue culture centre	American testing centre and culture	American type culture collection	Association of tissue culture collection	American type culture collection
What is the expanded form of MCC?	Centre for microbial	Microbial culture	Microbial cell culture	Microbs collection centre	Microbial culture collection
The organism which grows best above 45°C are called_____	psychrophilic	mesosphilic	thermophilic	hygrophiles	thermophilic
The organism which grows best below 15°C are called___	psychrophilic	mesosphilic	thermophilic	hygrophiles	psychrophilic
The organism which grows best between 25°- 45° C are called _____	psychrophilic	mesosphilic	thermophilic	hygrophiles	mesosphilic
Which of the following is used to grow bacterial cultures continuously?	chemostat	coulter counter	hemostat	petroff-hausser chamber	chemostat
Some organisms can use reduced inorganic compounds as electron donors and are termed as	chemolithotrophs	phototrophs	chemorganotrophs	photo-organotrophs	chemolithotrophs
Some organisms can use reduced organic compounds as electron donors and	chemolithotrophs	phototrophs	chemorganotrophs	photo-organotrophs	chemorganotrophs
Some organisms can use solar energy are termed as	chemolithotrophs	phototrophs	chemorganotrophs	lithotrophs	phototrophs
The growth is normally expressed as _____ in turbidimetric	cells per ml	cfu/ml	optical density	mg n2 /ml	optical density
Bacteria and fungi multiply best at	below 16°c	between 16-38°c	above 38°c	above 100°c	between 16-38°c
Which of the following procedures uses a photocell to measure absorbance of a culture to regulate the flow of culture media?	coulter counter	hemostat	petroff-hausser chamber	trubidostat	trubidostat
Which of the following is the suitable temperature range for mesophiles?	20-30°C	25-40°C	>40°C	>100°c	25-40°C
Exponential phase of growth curve of bacteria is of limited duration because of	rise in cell density	accumulation of toxic metabolites	exhaustion of nutrients	rise cell density, toxic products and exceed nutrients	rise cell density, toxic products and exceed nutrients
In the exponential phase, the cells and cell mass	first increases then	decreases	are constant	double at a constant rate	double at a constant rate
The period between inoculation of bacteria in a culture medium and beginning of multiplication is known as	stationary phase	log phase	lag phase	decline phase	lag phase
A common filter medium is the cloth filter generally made of	canvas	synthetic fabrics	metal or glass fiber	all of these	all of these
Which of the following is incorrect?	strain should be pure, and free from phage.	strain should be genetically stable, but amenable to genetic	strain produce a single valuable product, and no toxic by-products.	the option a, b and c is wrong for choosing microbes	the option a, b and c is wrong for choosing microbes
Which of the following is correct?	strain should be pure, and free from phage.	strain should be genetically non stable.	strain Should produce multiple products	strain Should produce toxic by-products.	strain should be pure, and free from phage.
How many types of streaking are there?	2	3	4	1	3
The book entitle '*Studies on Fermentation*' published by ____	Louis Pasteur	Eduard Buechner	Arthur Harden	Hesseltine	Louis Pasteur
What is biomass ?	weight of living	colony	dead matters	dead cells	weight of living organism
Name the location of microbial culture collection (MCC) in India?	Delhi	Pune	Chandigarh	Chennai	Pune
What is the expanded form of ATCC?	American tissue culture centre	American Type Culture Collection	American tissue and culture collection	american culture collection centre	American Type Culture Collection
A culture in which only one strain or clone present is called ……	Colony culture	Pure culture	Enrichment culture	mixed culture	Pure culture
What is the way to find antibiotic producing organism?	colour pigments around the colony	clear zone around the colony	white crystals around the colony	green crystals around the colony	clear zone around the colony
. …….. is used for to identify the lactic acid producing microorganism	sodium chloride	calcium carbonate	calcium nitrate	KOH	calcium carbonate
What is the way to find lactic acid producing organism?	colour pigments around the colony	clear zone around the colony	white crystals around the colony	green crystals around the colony	white crystals around the colony
What is homofermentative culture ?	organism produces only one metabolites	organism produced more than one metabolites	organism produced more than two metabolites	organism produced more than three metabolites	organism produces only one metabolites
Improving the quality of microbial strains artificially is called ……..	strain improvement	pure culture	colony	mixed culture	strain improvement
What is heterofermentative culture ?	organism produces only one metabolites	organism produced more than one metabolites	organism produced more than two metabolites	organism produced more than three metabolites	organism produced more than one metabolites
The detection of only those microorganisms which are of interest from among a large microbial population are known as _____	isolation	strain improvement	screening	selection	screening
What is the use of bromothymol blue in microbial screening?	antibiotic producing microbes	Growth factor producing microbes	Organic acid producing microbes	enzyme producing microbs	Organic acid producing microbes
……. used as a pH indicating dye	crystal violet	neutral red	safranin	violet	neutral red
Who was introduced the enrichment culture technique?	Louis Pasteur	Eduard Buechner	Arthur Harden	Martinus Beijerinck	Martinus Beijerinck
Calcium carbonate reacts with lactic acid and form ……. as byproduct	calcium chloride	calcium lactate	cabonic acid	lactic acid	calcium lactate

What is the expanded form of MIC?	Microbial inhibition concentration	Mass inhibitory culture	MIC	inhibition	Microbial inhibition concentration
_____ technique used for identification of growth factor producing	Enrichment	Auxanography	crowded plate	dentrogram	Auxanography
The loss of a purine base (A or G) to form an apurinic site (AP site) is known	Deamination	Depurination	Tautomerism	purination	Depurination
The exchange of single nucleotide with another one nucleotide sequence are known as ____	Point mutation	deletion	substitution	addition	Point mutation
A base is replaced by one of the other three bases are known as _____	Substitution	Point mutation	deletion	addition	Substitution
_____ enzyme used to cut the plasmid DNA	DNA Ligase	restriction enzyme	lipase	chitinase	restriction enzyme
What is meant by solid state fermentation?	Growing of microbes on moist solid particles or surface	Growing of microbes on nutrient broth	Growing of microbes on solid and liquid	Growing microbes on water	Growing of microbes on moist solid particles or surface
Separation of a compound from a liquid mixture by treatment with a solvent resulting in solubility of desired component _____	liquid-solid extraction	liquid-liquid extraction	liquid-gas extraction	solid-liquid extraction	liquid-liquid extraction
Unit III					
Which device used to monitor the pH when operating the bioreactor ?	Thermocouple	Calomel electrode	Mercury in glass	electrode	Calomel electrode
Which device used to monitor the temperature when operating the bioreactor ?	Thermocouple	Calomel electrode	both	diaphrahm	Thermocouple
Integrated device with fermenter and operated by computer directly are known as_____	In line sensor	off line sensor	online sensor	on/off sensor	online sensor
Integrated device with fermenter and operated by computer indirectly are known as_____	In line sensor	off line sensor	online sensor	on/off sensor	In line sensor
Which parts used to reduce the vortex?	Baffle	agitator	impeller	shaft	Baffle
The fermenter vessel should made up of _____	Iron steel	stainless steel	aluminium	chromium	stainless steel
The fermenter vessel should not made up of _____	Iron steel	stainless steel	copper	chromium	Iron steel
How many number of baffle will there in a fermenter ?	5	7	4	10	4
Which device used to introduce the air to the fermenter	Baffle	sparger	impeller	agitator	Baffle
What is on line sensor?	It is a sensor integrated with machine directly control the fermenter	It is a sensor non integrated with machine directly control the	non integrated	manual operation	It is a sensor integrated with machine directly control the fermenter
What is off line sensor	It is a sensor integrated with machine directly control the fermenter	It is a sensor non integrated with machine directly control the	non integrated	manual operation	It is a sensor non integrated with machine directly control the fermenter
When foam formation is high _____ solution may be add to reduce the	Precursor	elicitor	antifoam agent	media	antifoam agent
How you will reduce the heat if heat is exceed in a vessel?	Water jacket	Heating coil	heater	exchanger	Water jacket
How you will increase the heat if heat is exceed in a vessel?	Water jacket	Heating coil	coolent	exchanger	Heating coil
Which buffer will use to increase the pH?	Acidic buffer	base buffer	acid	alakaline	base buffer
_____buffer will use to decrease the pH?	Acidic buffer	base buffer	acid	alakaline	base buffer
_____ type of impeller are there?	5	3	4	6	4
How you maintain aeration in a vessel ?	through operating agitator	through operating stirrer glands	through sparger	through screw	through operating agitator
How monitor the pressure and which device will use to maintain?	C bourden pressure	pressure diaphram	through sparger	C bourden pressure guage and diaphram	C bourden pressure guage and
_____ device used to monitor the flow	Guage	rota meter	diaphram	rotor	rota meter
What is expanded form of PID?	Proportional, integral and derivative	Pump induction device	diaphram	rotor	Proportional, integral and derivative
What is the use of operation of impeller?	Uniform mixing of the	Uniform mixing of	Uniform mixing of Co2,	None of the above	Both
Foam formation in a vessel due to the presence of _____	Carbohydrate	protein	enzyme	fat	protein
How many types of sparger are there?	1	2	3	4	3
Fermenter is under the direct control of the computer software are called	DDC	SSC	SSPC	CSTR	DDC
Fermenter is under the indirect control of the computer software are called	DDC	SSC	SSPC	CSTR	SSC
What is the expanded form of DDC?	Direct digital control	direct digital circuit	digital circuite	control panel	Direct digital control
What is expanded form of SSC?	super system control	set point control	supervisory set point	manual operation	supervisory set point control
What is abbreviated form of Direct digital control?	DDC	DC	SSC	CSTR	DDC
What is abbreviated form of supervisory set point control?	SSPC	SP	SSC	CSTR	SSC

What is the use of sparger?	Introduction of air to the vessel	Introduction of liquid flow to the vessel	Introduction of solid flow the vessel	Introduction of media to the vessel	Introduction of air to the vessel
The place of arrangement of agitator are known as _____	agitator holder	agitator shaft	holder	bearings	agitator shaft
In calomel electrode the sensor made of _____	calcium chloride	silver chloride	copper	iron	silver chloride
_____ are surface active agents reducing the surface tension in the foams and destabilizing protein films.	detergents	foams	antifoams	surfactants	antifoams
The function of the disengagement zone in an airlift fermenter is to	prevent co2 rich bubbles from entering the downcomer	reduce the velocity of the bubbles	reduce liquid loss as aerosols	prevent co2 rich bubbles from entering the downcomer, reduce the velocity of the bubbles, reduce liquid loss as	prevent co2 rich bubbles from entering the downcomer, reduce the velocity of the bubbles, reduce
The excess Co2 inside the vessel cause _____	BOD	COD	DO	catalase	BOD
High speed operation of agitator cuase _____	Cell death	cell growth	increase biomass	decrease biomass	Cell death
Open and closing device in reactor are called _____	impeller	shaft	valves	screw	valves
_____ device used for monitor the pressure	pressure gauge	valves	screw	shaft	pressure gauge
What is the disadvantage of foam in a vessel?	Reduce o2 transfer	Reduce the nutrient transfer and dissolve	Both	reduce the growth level	Both
What is antifoam?	Agent that to reduce the foam formation	Agent that to produce the foam	Agent that generate heat	agent to generate pressure	Agent that to reduce the foam formation
What is the role of heating coil?	Generate heat or temperature	Increase mass	Increase pH	Gives cool	Generate heat or temperature
Widely used sterilization method for fermentation media are _____	Dry heat sterilization	Chemical sterilization	Steam sterilization	surface sterilization	Steam sterilization
What is Rheology?	study of flow and deformation of materials under applied forces.	Study of pH and its role	Study of temperature in fermentation	study of pressure in reactor	study of flow and deformation of materials under applied forces.
Flow and deformation of materials under applied forces are known as	Kinetics	Rheology	isolation	screening	Rheology
What is Kinetics ?	The study of motion and chemical reaction rates in fermentation	study of flow and deformation of materials under applied forces.	study of reactor	study of pH	The study of motion and chemical reaction rates in fermentation
What is Mass transfer?	The net movement of mass (nutrient) from one location to another in a	Rate of chemical reaction	movement of water	movement of air	The net movement of mass (nutrient) from one location to another in a vessel.
What is energy transfer?	Rate of chemical reaction	Exchange of energy from one place to another place in s vessel.	Motion of matter	exchange nuitrient	Exchange of energy from one place to another place in s vessel.
To increase the pH, _____ solution to be added	acidic buffer	Basic buffer	acids	salts	Basic buffer
The diffusion or transfer of oxygen from the bubbles in the broth phase to the bulk liquid phase.	Massy transfer	Heat transfer	Film theory	reology	Film theory
Metafilters are primarily used for _____ liquids such as beer.	foaming	precipitating	polishing	separating	polishing
The process of recovery and purification of fermentation products is called _____ processing.	downstream	upstream	central	recovery	downstream
Microbial cells and other insoluble materials are normally separated from the harvested broth by _____.	Precipitation	cell disruption	foaming	filtration	filtration
Materials that are made surface active are called as _____.	Collectors	colligends	reactants	separators	colligends
_____ can be precipitated out of a broth by the addition of methanol.	acetone	dextran	starch	ethanol	dextran
_____ has been obtained from *S. cerevisiae* by freeze thawing.	glucosidase	alpha-glucosidase	gamma-glucosidase	beta-glucosidase	beta-glucosidase
The frequency of vibration used in ultrasonication for cell disruption is	@ 10 kHz	@ 20 kHz	@ 30 kHz	@25 kHz	@ 20 kHz
Fluids above their critical temperature and pressure are called as _____.	supernatural fluids	critical fluids	supercritical fluids	natural fluids	supercritical fluids
The common cation exchange resin is _____.	carboxy methyl cellulose	methyl cellulose	cellulose	DEAE	carboxy methyl cellulose
A common anoin exchange resin is _____.	carboxymethyl cellulose	diethylamioethyl	amberlite	PAE	diethylamioethyl cellulose
Unit IV					
Protease, which is used for flavouring of sake and haze removal in sake is	*A. oryzae*	*A. flavus*	*B. cereus*	*A. niger*	*A. oryzae*
Penicillin G is also known as	hydroxy benzyl penicillin	phenoxy methyl	benzyl penicillin	2-pentenyl penicillin	benzyl penicillin
A major organic acid produced by a microbial process used in foods is	sulfuric acid	citric acid	oxalic acid	uric acid	citric acid

In aerobic respiration, the terminal electron acceptor is	oxygen	nitrogen	hydrogen	nitrate	oxygen
The reactions of the cell that are carried out for capturing energy are called	catabolism	metabolism	anabolism	activation energy	catabolism
_____ is an enzyme which is bound to the outer membrane of *Klebsiella*	pullulanase	pectinase	protease	amylase	pullulanase
_____ caused by a sudden change in salt concentration will cause disruption of a number of cell types.	liquid shear	solid shear	osmotic shock	detergents	osmotic shock
Osmotic shock has proved to be successful technique for the extraction of _____ from *Phototbacterium fischeri*	luciferase	pectinase	amylase	cellulase	luciferase
_____ is an antibiotic which is recovered from fermentation broths by centrifugal counter-current solvent extraction.	streptomycin	penicillin-G	griseofulvin	nystatin	penicillin-G
One early industrial application of gelpermeation method was the purification of _____.	organic compounds	proteins	vaccines	carbohydrates	vaccines
Agarose activated with _____ is one of the most commonly used supports for the coupling of aminoligands.	sodium bromide	calcium bromide	cyanogen bromide	potassium ioide	cyanogen bromide
____ is a process in which solutes of high molecular weight pass through a fine pore sized membrane under hydraulic pressure	filtration	ultrafiltration	precipitation	centrifugation	ultrafiltration
_____ is most widely used for drying of biological materials when the starting material is in the form of a liqud orpaste.	drum driers	fluidized bed driers	spray driers	freeze driers	spray driers
_____ are increasingly used in the pharmaceutical industry.	drum driers	fluidized bed driers	spray driers	freeze driers	fluidized bed driers
An established method used in the initial recovery of organic acids and aminoacids is _____.	crystallization	filtration	centrifugation	drying	crystallization
___ is the quantity of oxygen required for the oxidation of organic matter by microbes present in given temperature/time.	COD	BOD	oxygen demand	chemical demand	BOD
The normal BOD strength of domestic sewage is _____.	320 mg dm^{-3}	350mg dm^{-3}	300mg dm^{-3}	290mg dm^{-3}	350mg dm^{-3}
_____ is a disposal method for municipal solid waste and industrial	oxidation pond	lagoons	landfilling	incineration	landfilling
The removal of suspended solids by physical methods before biological treatment will _____ BOD of the resulting effluent.	increase	decrease	double	concentrate	decrease
The commonly used coagulant for chemical teatment of fermentation wastes is	sodium carbonate	sodium sulphate	aluminium hydroxide	aluminium sulphate	aluminium sulphate
_____ is a high resolution column chromatographic technique.	HPLC	RPC	ion-exchange	adsorption	HPLC
_____ invovles binding of the solute to the solid phase primarily by weak vander waals forces.	ion exchange	adsorption chromatography	affinity chromatography	continuous chromatography	adsorption chromatography
_____ can be extracted from filtrates using activated charcoal columns.	streptomycin	mono-hydrostreptomycin	di-hydrostreptomycin	penicillin	di-hydrostreptomycin
_____ is the removal of solvent from a solution by the applicatin of heat to	evaporation	distillatin	condensation	evacuation	evaporation
Alkali treatment of the fermentation broth is useful in the extraction of	D- asparaginase	L-asparaginase	luciferase	streptoginase	L-asparaginase
_____ centrifuge is used for continuous handling of fermentation	multichamber	solid-bowl scroll	basket	tubular bowl	solid-bowl scroll
A centrifuge to consider using for particle size ranges of 0.1 to 200 μm and up	multichamber	tubular bowl	continous	basket	tubular bowl
The use of _____ is used in the effluent treatment industries for the removal of	flocculating agents	salting agents	sodium salts	calcium salts	flocculating agents
_____ is the movement of component molecules in a mixture under the influence of a concentration difference in the system.	dilution	molecular diffusion	molecular conduction	convention	molecular diffusion
The yeast generated during the fermentation of beer is generally separated by	centrifugation	filtration	cell disruption	coagulation	centrifugation
Final alcohol content in wine varies from	6-9 % by weight	10-20 % by weight	30-40 % by weight	40-50 % by weight	6-9 % by weight
What is the desirable sugar content of the grapes required for the wine	2-5%	5-10%	10-14%	14-20%	14-20%
The process of making malt as soluble as possible by using enzymes adjuncts etc is known as	brewing	malting	mashing	pitching	mashing
Wort is	an aqueous extract of malt	malted barley	coagulated protein obtained during boiling	baggas	an aqueous extract of malt
The germination of barley kernels under controlled temperature and humidity to generate enzymes for the degradation of starch and protein is known as	brewing	malting	mashing	pitching	malting
Wort is boiled with the hops	to help in protein coagulation	to provide bitter taste and flavour to the beer	to help in the coagulation of tannins	act as surfactant	to provide bitter taste and flavour to the beer

_____ has been used as the major nitrogen source in a defined medium for the commercial production of human serum albumin by Saccharomyces	nitrate	nitrite	ammonia	protein	ammonia
In aerobic yeast fermentation for production of citric acid from alkanes using a fed-batch culture, why alkanes are slowly fed to the yeast?	Citric acid is toxic to the cells	Alkanes cause foaming	Fast addition of alkanes will inhibit the cells and reduce oxygen transfer rates	Fast addition of alkanes will cause the cells to grow too quickly	Fast addition of alkanes will inhibit the cells and reduce oxygen transfer rates
Which of the following is used to calculate mass of substrate in the reactor?	Flow rate x substrate concentration in the reactor	Volume of reactor x substrate concentration in reactor	Flow rate x mass of reactor	Volume of reactor x Flow rate	Volume of reactor x substrate concentration in reactor
Low dissolved oxygen concentrations leads to	low biomass yields	high biomass yields	no effect on biomass yields	equal biomass	low biomass yields
The main reason for production of antibiotics in fed batch reactors is	the presence of precursors is often toxic	reduce heat	reduce the cool	increase temp	the presence of precursors is often toxic to the cells
A fed-batch reactor initially contains 2 litre of medium. If it was fed at 1 litre per hour, then after 10 hours, the volume of the reactor will be	1 litre	2 litre	3 litre	13 litre	2 litre
The function of the disengagement zone in an airlift fermenter is to	prevent co2 rich bubbles from entering the downcomer	reduce heat	reduce the cool	increase temp	prevent co2 rich bubbles from entering the downcomer
Stationary phase is described as	no further increase in the cell population after a maximum value	deceleration of growth and division rate after the growth rate reaches a	acceleration of growth and division rate after the growth rate reaches a	deceleration of growth and division rate after the growth rate reaches a minimum	no further increase in the cell population after a maximum value
In the accelerated phase, cell starts to	increase and the division rate increases to reach a maximum	decrease and the division rate increases to reach a maximum	increase and the division rate decreases to reach a maximum	increase and the division rate increases to reach a minimum	increase and the division rate increases to reach a maximum
Why a T-flask used in small-scale cell culture is incubated in a horizontal position?	to save space	to increase the surface area of the liquid-air interface	To save space and increase the surface area of the liquid-air interface	to increase the rate of oxygen transfer into the liquid	To save space and increase the surface area of the liquid-air interface
Fermentor should be filled with medium upto	65-70%	70-75%	75-80%	80-85%	75-80%
Which of the following was the first amino acid to be produced commercially?	L-glutamic acid	L-lysine	L-cystine	L-methionine	L-lysine
The first penicillin isolated by Alexander Fleming, penicillin F is also called	2-Pentenyl Penicillin	n-heptyl penicillin	phenoxymethyl penicillin	benzyl penicillin	2-Pentenyl Penicillin
Pectinase, an enzyme used for increasing yield and for clarifying juice is	*A. oryzae*	*Saccharomyces*	*Penicillium chrysogenum*	*bacillus*	*A. oryzae*
Which species from the followings is resistant to methyl tryptophan?	*Candida utilis*	*E. coli*	*B. subtilis*	*Hansenula anomala*	*B. subtilis*
Which of the following substance is employed to neutralize the lactic acid as it is produced (because lactic acid bacteria do not tolerate high concentrations of	CaCO3	(NH4)2 HPO4	MgSO4	Na2SO4	CaCO3
Alcohol production from starch and raw sugar utilizes selected strains of	*Saccharomyces*	*Candida*	*Candida utilis*	*bacillus*	*Saccharomyces cerevisiae*
Fungal amylases using stationary culture with wheat bran utilizes	*A. oryzae*	*A. niger*	*A. flavus*	*S. cerevisiae*	*A. oryzae*
Why high concentration of sugars are not employed in the fermentation medium?	Calcium lactate is not produced	Calcium lactate crystallizes from the medium and slows down the process of	High sugar concentration itself crystallizes	Low sugar concentration	Calcium lactate crystallizes from the medium and slows down the process of fermentation
_____are commonly used by industries which produce large volumes of liquid which needs continuous processing.	batch filters	rotary vacuum filters	plate filters	frame filters	rotary vacuum filters
_____ centrifuges are useful for separating mould mycelia or crystalline	tubular bowl	solid bowl	basket	disc	basket
_____ is the method which has been most widely used in large scale enzyme purification processes.	solid shear	liquid shear	alkali treatment	osmotic shock	liquid shear
_____ is a method of cell disruption that might be ideal for microbial product which are very temperature lable.	liquid shear	detergents	solid shear	ultrasonication	solid shear
Yield coefficient represents	total biomass or product produced	conversion efficiency of a substrate into product	conversion rate of a substrate into biomass or	production time of biomass or product	conversion efficiency of a substrate into product
UNIT V					
_____Which organism widely used for the production of lactic acid	*Aspergillus*	*Lactobacillus*	*Acetobactor*	*bacillus*	*Lactobacillus*
What is the source of penicillin?	*Penicillium*	*Aspergillus niger*	*Saccharomyces*	*bacillus*	*Penicillium chrysogenum*
_____ organism used for ethanol production	*Aspergillus*	*Saccharomyces*	*Penicillium chrysogenum*	*bacillus*	*Saccharomyces*

Yield coefficient represents	total biomass or product produced	conversion efficiency of a substrate into product	conversion rate of a substrate into biomass or	production time of biomass or product	conversion efficiency of a substrate into product
Microbial cells and other insoluble materials are normally separated from the harvested broth by _____.	Precipitation	cell disruption	foaming	filtration	filtration
Materials that are made surface active are called as _____.	Collectors	colligends	reactants	separators	colligends
_____ can be precipitated out of a broth by the addition of methanol.	acetone	dextran	starch	ethanol	dextran
Non-ionic polymers such as _____ can be used in the precipitation of	PEG	glycol	ethylene	PGE	PEG
The most commonly used filter aid is _____.	kiesulghur	filterpaper	salts	acids	kiesulghur
Metafilters are primarily used for _____ liquids such as beer.	foaming	precipitating	polishing	separating	polishing
_____ has been obtained from *S. cerevisiae* by freeze thawing.	glucosidase	alpha-glucosidase	gamma-glucosidase	beta-glucosidase	beta-glucosidase
Which of the following is not the physical method for the cells rupturing?	milling	homogenization	ultrasonication	enzymatic digestion	enzymatic digestion
If the solute passes through membrane freely, the rejection coefficient (s) is	1	0	>1	<1	0
The method or processes for releasing biological molecules from inside a cell are known as _____	Motion	Cell disruption	Heat transfer	reaology	Cell disruption
Mostly Cell disruption method used for _____	To release intra cellular components	To release extra cellular components	Extra and intra compounds	secondary metaboltes	To release intra cellular components
_____ involves the freezing of a culture followed by its drying under	drying	crystallization	lyophilization	refrigeration	lyophilization
_____ are surface active agents reducing the surface tension in the foams and destabilizing protein films.	detergents	foams	antifoams	surfactants	antifoams
A continuous bioreactor in which only the flow rate is used to control the rate of cell or product productivity is called	turbidostat	chemostat	level stat	pH	chemostat
Recovery and purification of biosynthetic products from fermented media are known as ____	Upstream process	isolation	downstream process	seperation	downstream process
In a liquid solution, settle down the solid substances at the bottom of the vessel are called _____	Coagulation	filtration	precipitation	purification	precipitation
In fermented liquid media, precipitate the solid substances at the bottom of the vessel are called _____	Coagulation	filtration	sedimentation	purification	sedimentation
In a liquid solution, settle down the solid substances at the bottom of the vessel due _____	Coagulation rate	gravitational force of the particles filtration	affinity of the particle	surfactant	affinity of the particle
Process that formation of crystal of solute or compound present in the liquid are known as _____	Coagulation	filtration	precipitation	crystallization	crystallization
Bead mill of cell disruption method also known as _____	Chemical method	mechanical method	physical	biological	mechanical method
Liquid shear of cell disruption method also known as _____	mechanical method	Chemical method	physical	biological	mechanical method
_____ will be add as precipitating agent	sodium chloride	Sodium hydroxide	Alum	sulfate	Alum
In frame filter, the filter cake made up of _____	Plaster of paris	diatomaceous earth	cellulose	platic	diatomaceous earth
Transferring the one or more solute containing feed solution to another immiscible liquid (solvent) for separation are known as _____.	Elution	fractionation	liquid- liquid extraction	gel permeation	liquid- liquid extraction
The region of an airlift bioreactor in which the liquid travels in a downward direction is called the	downcomer	disengagement zone	air riser	flotsam	downcomer
A system which require less solvent and produces a more concentrated extract phase, is desired with a	large distribution coefficients	small distribution coefficients	very small distribution coefficients	constant distribution coefficients	large distribution coefficients
The efficiency of cell disruption in a bead mill depends on the	concentration of the cells	amount and size of beads	type and rotational speed of agitation	concentration of the cells, size of beads, type and rotational speed of agitation	concentration of the cells, size of beads, type and rotational speed of
An ion exchange resin is composed of	polymeric network	ionic functional groups	counter ions	Polymeric network, ionic functional groups, counter ions	Polymeric network, ionic functional groups, counter ions
The disk centrifuge is the type of centrifuge used most often for bio separations	continuous operation	lesser cost	higher speed	ease in operation	lesser cost
The stage wise operation of adsorption is called	contact filtration	conventional adsorption	affinity adsorption	ion exchange	contact filtration
Conventional adsorption is a	reversible process	irreversible process	either reversible or	Equavalency	reversible process
In antibiotic manufacturing processes, the fermentation time ranges from	2-3 weeks	1-2 weeks	4-5 weeks	2-4 weeks	4-5 weeks
Which of the operation does not come under upstream processing?	Media preparation	Inoculum development	Effluent treatment	Storage of raw material	Effluent treatment
Micro filtration refers to the separation of suspended material such as bacteria by using a membrane with spore sizes of	0.02 to 10µm	1-10A°	20-30µm	10-200A°	0.02 to 10µm

The purity of a solute collected between two times t1 and t2 during chromatographic separation can be calculated as	amount of solute eluted - amount of impurity	amount of solute eluted / amount of impurity	amount of solvent eluted + amount of impurity eluted	amount of solvent eluted / amount of impurity eluted	amount of solute eluted / amount of impurity eluted
Chromatography is based on the	different rate of movement of the solute in the column	separation of one solute from other constituents by being captured on the	different rate of movement of the solvent in the column	separation of compounds	different rate of movement of the solute in the column
Which of the following organism produces diastase enzyme?	*A. oryzae*	*B. subtilis*	*A. niger*	*S. cerevisiae*	*A. oryzae*
The biotin level in obtaining L-gluatmic acid by fermentation using Micrococcus glutamicus is critical because	low level prevents growths, hence L-glutamic acid production	high level prevents L-glutamic acid production	no effects	balance the production rate	high level prevents L-glutamic acid production
Most productive species among the different vitamin B12 producing	*P. denitrificans*	*P. ovalis*	*P. aureofaciens*	*Bacillus*	*P. denitrificans*
The enzyme which converts optically active isomers of lactic acid to the optically inactive racemic mixture is	isomerase	racemase	dehydrogenase	invertase	racemase
The sugar concentration of molasses used in fermentation should be	10-18%	20-30%	4-5%	30-38%	10-18%
Bacitracin, an antibiotic similar to penicillin, is produced by	*Bacillus polymyxa*	*Streptomyces erythreus*	*Bacillus licheniformis*	*Aspergillus fumigates*	*Bacillus licheniformis*
Which of the following Ascomycetes are used for riboflavin production?	*Eremnothecium ashbyii*	*Ashbya gossypii*	*Candida guilliermondia*	Both (a) and (b)	Both (a) and (b)
B. flavum strain can be used for the production of	L-Threonine	galactase	galactate	gallic acid	L-Threonine
A major organism used in commercial bioleaching for copper recovery is	*Desulfovibrio desulfuricans*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Thiobacillus ferrooxidans*	*Thiobacillus ferrooxidans*
During fermentation, pyruvic acid is converted into organic products such as	glucose and fructose	starch and cellulose	ethyl alcohol and lactic acid	citric acid and isocitric acid	ethyl alcohol and lactic acid
Which of the following are true for the heterofermentative microorganism producing lactic acid?	Only trace amounts of end products other than lactic acid	Some lactic acid with carbon dioxide, ethyl alcohol, acetic acid and trace amount of few	They are used for commercial lactic acid production	used for food preservation	Some lactic acid with carbon dioxide, ethyl alcohol, acetic acid and trace amount of few other products
The submerged fermentation of vinegar utilizes special fermentor designs	acetator	cavitator	packed vinegar generator	acetator and cavitator	acetator and cavitator
The major organism used in the microbial production of citric acid is	*Penicillin notatum*	*Rhizopus nigrificins*	*Aspergillus niger*	*Lactobacillis delbrueckii*	*Aspergillus niger*
Which of the following organism is utilized for the production of D-L-alanine?	*Microbacterium ammoniaphilum*	*Brevibacterium flavum*	*Arthrobacter paraffineus*	*C. glutamicum*	*Microbacterium ammoniaphilum*
The aerated submerged bacterial fermentation to produce vitamin B12 employs	*Propionibacterium*	*bacillus*	*Acetobacter*	Penicium	*Propionibacterium*
Which of the following is a heterofermentative micro-organism?	*Leuconostoc mesenteroides*	*Lactobacillus delbrueckii*	*Lactobacillus bulgaricus*	*Lactobacillus pentose*	*Lactobacillus delbrueckii*
Riboflavin is a by-product of _____ fermentation carried out by	citric acid	acetone butanol	streptomycin	ethanol	acetone butanol
The organism which utilizes the pentose of sulphite waste liquor for lactic acid	*Streptococcus lactis*	*Lactobacillus bulgaricus*	*Lactobacillus casei*	*Lactobacillus pentosus*	*Lactobacillus pentosus*
The sugar concentration of molasses used in fermentation should be	10-18%	20-30%	4-5%	30-38%	10-18%
The recovery of vitamin from fermentation broth is carried out prior to	acidification	alcohol treatment	autolysis	alkaline teratment	autolysis